Vol. 16. No. 1. FEBRUARY, 1961

BRITISH JOURNAL OF PHARMACOLOGY AND CHEMOTHERAPY

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Brit. J. Pharmacol. (1961), **16**, 1–5.

PROCEEDINGS OF THE BRITISH PHARMACOLOGICAL SOCIETY

ROYAL COLLEGE OF SURGEONS OF ENGLAND, LONDON

5th to 7th January, 1961

COMMUNICATIONS

P. B. Bradley and **E. Marley** (*Department of Experimental Psychiatry, The Medical School, Birmingham*, 15).

Effects of tryptamine and some tryptamine analogues on cerebral electrical activity.

E. Marley and **J. R. Vane** (*Department of Pharmacology, Royal College of Surgeons of England, London, W.C.*1).

The effects of some tryptamines on spinal reflexes.

S. J. Corne (*Department of Pharmacological Research, Parke, Davis & Co., Hounslow, Middlesex*).

Some actions of tryptamine and its homologues α-methyltryptamine and α-ethyltryptamine.

G. V. R. Born and **J. R. Vane** (*Department of Pharmacology, Royal College of Surgeons of England, London, W.C.*1).

The penetration of labelled tryptamine and 5-hydroxytryptamine into smooth muscle.

J. Thuillier (*introduced by* **P. B. Bradley**) (*Clinique des Maladies Mentales, Faculté de Médecine, Paris*).

Pharmacological aspects of dimethylaminoethylic ester of *p*-chlorophenoxyacetic acid (centrophenoxine).

H. O. J. Collier and **B. T. Warner** (*Department of Pharmacological Research, Parke, Davis & Co., Hounslow, Middlesex*).

A multiple toe-pinch method of evaluating analgesics.

N. Ambache and **M. Reynolds** (*Department of Physiology and Medical Research Council, Royal College of Surgeons of England, London, W.C.*1).

Differences in fatty acid receptors.

Barbara Boughton and **H. O. Schild** (*Pharmacological Laboratory, University College, London, W.C.*1).

Passive transfer of delayed hypersensitivity in testis autosensitization.

K. A. P. Edman and **J. L. Mongar** (*Pharmacological Laboratory, University College, London, W.C.*1).

The essential role of S-H and S-S groups in the anaphylactic reaction.

K. A. P. Edman and **H. O. Schild** (*Pharmacological Laboratory, University College, London, W.C.*1).

Effects of calcium in depolarized smooth muscle.

P. Acred, D. M. Brown, D. H. Turner and **D. Wright** (*Research Division, Beecham Research Laboratories, Betchworth, Surrey*).

Absorption, distribution and elimination of 2: 6-dimethoxyphenyl penicillin (B.R.L. 1241).

P. F. D'Arcy and **E. M. Howard** (*Research Division, Allen & Hanburys, Ware, Herts*).

The relative acute oral toxicity of ferrous salts in dogs.

R. T. Brittain, H. O. J. Collier and **P. F. D'Arcy** (*Research Division, Allen & Hanburys, Ware, Herts*).
Some observations on γ-oxalolaudonium bromide—a new neuromuscular blocking drug.

A. L. A. Boura and **A. F. Green** (*The Wellcome Research Laboratories, Beckenham, Kent*).
Some effects of adrenergic neurone blockade on responses to sympathomimetic amines.

M. F. Sim and **A. F. Green** (*The Wellcome Research Laboratories, Beckenham, Kent*).
The effects on diuresis in rats of compounds that modify sympathetic actions.

Doreen M. Anderson and **W. G. Smith** (*Pharmacy Department, Sunderland Technical College, Co. Durham*).
The antitussive activity of glycyrrhetinic acid and some of its water-soluble derivatives.

J. M. Thorp (*Pharmaceuticals Division, Imperial Chemical Industries, Macclesfield, Cheshire*).
Seasonal variation: a factor in the interpretation of certain biological tests.

J. Raventós (*Pharmaceuticals Division, Imperial Chemical Industries, Macclesfield, Cheshire*).
On halothane hypotension.

A. Spinks (*Pharmaceuticals Division, Imperial Chemical Industries, Macclesfield, Cheshire*).
Rapid colorimetric estimation of monoamine oxidase and 5-hydroxytryptophane decarboxylase.

M. J. Rand (*Department of Pharmacology, School of Pharmacy, London, W.C.*1).
The action of hemicholinium (HC3) on sympathetic nerve transmission.

J. M. Telford (*introduced by* **G. B. West**) (*Department of Pharmacology, The School of Pharmacy, London, W.C.*1).
Adrenal cortical steroids, histamine liberators and histamine formation in the rat.

J. M. Harris (*introduced by* **G. B. West**) (*Department of Pharmacology, School of Pharmacy, London, W.C.*1).
The anaphylactoid reaction in rats.

M. Gelfand and **G. B. West** (*Department of Pharmacology, School of Pharmacy, London, W.C.*1).
Studies with two methyl derivatives of lysergic acid.

A. R. Muir and **L. Golberg** (*Department of Anatomy, University of Edinburgh, and Benger Laboratories Ltd., Holmes Chapel, Cheshire*).
An electron microscopic study of the metabolism of iron-dextran.

A. W. Cuthbert and **L. C. Blaber** (*introduced by* **G. A. H. Buttle**) (*Department of Pharmacology, School of Pharmacy, London, W.C.*1).
Hen cholinesterases studied *in vitro*.

R. W. Ryall (*introduced by* **R. Wien**) (*May & Baker, Dagenham, Essex*).
Some effects of central nervous system stimulant drugs on the cat's nictitating membrane.

D. R. Maxwell, W. R. Gray and **E. M. Taylor** (*introduced by* **R. Wien**) (*May & Baker, Dagenham, Essex*).
The relative activity of some inhibitors of monoamine oxidase in potentiating the action of tryptamine *in vitro* and *in vivo*.

V. C. Abrahams and **S. M. Hilton** (*introduced by* **W. Feldberg**) (*National Institute for Medical Research, Mill Hill, London, N.W.7*).

Actions of decamethonium on sympathetic and parasympathetic neurones.

P. Withrington and **Eleanor Zaimis** (*Department of Pharmacology, Royal Free Hospital School of Medicine, London, W.C.*1).

Evaluation of local anaesthetic drugs.

J. D. Harry (*introduced by* **G. Brownlee**) (*Department of Pharmacology, University of London King's College, London, W.C.*2).

The effect of cooling, local anaesthetics and botulinum toxin on the ACh output of the transmurally stimulated isolated guinea-pig ileum.

G. W. Pennington and **N. Jackson** (*introduced by* **A. Wilson**) (*Department of Pharmacology and General Therapeutics, University of Liverpool*).

The detection of doping substances in racehorses.

F. R. Domer and **V. G. Longo** (*Istituto Superiore di Sanita, Rome*).

The electro-encephalographic effects of 5-hydroxytryptophan in rabbits.

Session on Clinical Pharmacology

D. R. Laurence and **R. E. Nagle** (*University College Hospital Medical School, London, W.C.*1).

Observations on the interaction of bretylium, adrenaline and noradrenaline.

D. Alexander, **W. W. Buchanan**, **J. Crooks** and **D. Koutras** (*Department of Medicine, Western Infirmary, Glasgow, and Department of Materia Medica and Therapeutics, University of Aberdeen*).

The effects of d-thyroxine on thyroid function in man.

P. T. Nowell, **Carol A. Scott** and **A. Wilson** (*Department of Pharmacology and General Therapeutics, University of Liverpool*).

The urinary excretion of neostigmine and pyridostigmine in patients with myasthenia gravis.

Pamela M. Huby and **C. W. M. Wilson** (*Department of Pharmacology and General Therapeutics, University of Liverpool*).

Drug administration and suggestibility.

H. Wilson (*Department of Pharmacology and General Therapeutics, University of Liverpool*).

Some observations on patients with phaeochromocytomas.

E. M. Glaser (*introduced by* **A. Herxheimer**) (*Department of Physiology, The London Hospital Medical College, London, E.*1).

Subjective effects in students of different doses of hyoscine hydrobromide by mouth.

DEMONSTRATIONS

W. C. Bowman, **M. J. Rand** and **D. Tulett** (*Department of Pharmacology, School of Pharmacy, London, W.C.*1).

An isolated nerve-muscle bath suitable for electrical recording.

A. W. Lessin and **M. W. Parkes** (*Roche Products, Welwyn Garden City, Herts*).

The quantitative estimation of stimulant activity in mice.

J. W. Thompson (*Department of Pharmacology, Royal College of Surgeons of England, London, W.C.*1).

The nictitating membrane of the cat as an isolated nerve-smooth muscle preparation.

Margaret Day and **J. P. Green** (*Department of Pharmacology, Royal College of Surgeons of England, London, W.C.*1).

The uptake and synthesis of amines by neoplastic mast cells in culture.

J. E. Gardiner (*Department of Pharmacology, Royal College of Surgeons of England, London, W.C.*1).

A method for increasing the sensitivity of the frog rectus to acetylcholine.

C. Schneider (*Department of Pharmacological Research, Parke, Davis & Co., Hounslow, Middlesex*).

The effects of morphine-like drugs in chicks.

J. G. Bainbridge and **K. E. Wiggins** (*introduced by* **David M. Brown**) (*Research Division, Beecham Research Laboratories, Betchworth, Surrey*).

An apparatus for recording blood pressure on the penoscillograph.

J. G. Bainbridge and **K. E. Wiggins** (*introduced by* **David M. Brown**) (*Research Division, Beecham Research Laboratories, Betchworth, Surrey*).

A simple method for recording respiration on the penoscillograph.

J. D. Haines (*introduced by* **G. W. Cambridge**) (*Wyeth Laboratories, Havant, Hants.*).

A simple inexpensive electro-mechanical valve.

P. Withrington and **Eleanor Zaimis** (*Pharmacology Department, Royal Free Hospital School of Medicine, London, W.C.*1).

A method for estimating local anaesthetic activity.

G. J. Cunningham, L. Bitensky, J. Chayen, P. Gahan and **A. A. Silcox** (*introduced by* **G. V. R. Born**) (*Department of Pathology, Royal College of Surgeons of England, London, W.C.*1).

Histochemical methods of investigating toxic effects in liver.

G. J. Cunningham, J. Chayen, L. Bitensky and **A. A. Silcox** (*introduced by* **G. V. R. Born**) (*Department of Pathology, Royal College of Surgeons of England, London, W.C.*1).

Studies of response of mitochondria to toxic agents.

G. J. Cunningham, L. Bitensky and **A. A. Silcox** (*introduced by* **G. V. R. Born**) (*Department of Pathology, Royal College of Surgeons of England, London, W.C.*1).

The metabolism of a carcinogen.

A. A. Barton (*introduced by* **G. V. R. Born**) (*Department of Anatomy, Royal College of Surgeons of England, London, W.C.*1).

Electron micrographs of degenerating nerve.

D. H. Tompsett (*introduced by* **G. V. R. Born**) (*Department of Anatomy, Royal College of Surgeons of England, London, W.C.*1).

Casts of the cerebral circulation (cat, dog and human).

R. A. Butler (*introduced by* **G. V. R. Born**) (*Department of Anaesthetics, Royal College of Surgeons of England, London, W.C.*1).

The estimation by gas chromatography of low concentrations of volatile anaesthetics in gas mixtures and in tissue fluids.

D. W. Hill (*introduced by* **G. V. R. Born**) (*Department of Anaesthetics, Royal College of Surgeons of England, London, W.C.*1).

The estimation of halothane, diethyl ether and carbon dioxide by infra-red analysis.

H. Ghaleb (*introduced by* **G. A. H. Buttle**) (*School of Pharmacy, Brunswick Square, London, W.C.*1).

Metabolism of stilboestrol of the effects of prolonged administration in the hamster.

H. Ghaleb (*introduced by* **G. A. H. Buttle**) (*School of Pharmacy, Brunswick Square, London, W.C.*1).

Metabolism of the bi-phosphate ester of stilboestrol in carcinoma of the prostate.

Brit. J. Pharmacol. (1961), **16**, 6–14.

CARDIOVASCULAR ACTIONS OF PHENOXYBENZAMINE

BY

B. G. BENFEY

From the Department of Pharmacology, McGill University, Montreal, Canada

(*Received July* 13, 1960)

Phenoxybenzamine increased the force of contraction and rate of the heart and lowered the blood pressure of dogs in pentobarbitone anaesthesia. This was preceded by depression of cardiac contractile force and a rise in blood pressure when phenoxybenzamine was injected following ganglion-blocking drugs or atropine. The positive inotropic and chronotropic and the hypertensive effects of phenoxybenzamine were prevented by pretreatment with reserpine or guanethidine.

It has previously been found that phenoxybenzamine increases the concentration of adrenaline and noradrenaline in urine and plasma of dogs in barbiturate anaesthesia (Benfey, Ledoux & Melville, 1959; Benfey, Ledoux & Segal, 1959; Millar, Keener & Benfey, 1959). It was observed in these studies that the usual quick fall in blood pressure did not occur when the drug was injected during an infusion of hexamethonium. Further studies have now shown that it is possible to obtain a rise in blood pressure by injecting phenoxybenzamine following ganglion-blocking agents or atropine, and it appears that the drug is related to reserpine, which releases endogenous adrenaline and noradrenaline and lowers the blood pressure but has a transient hypertensive action when injected following ganglion-blocking drugs (Maxwell, Ross, Plummer & Sigg, 1957). However, this is hard to reconcile with the fact that phenoxybenzamine is an antisympathomimetic agent. Preliminary reports of parts of this work have appeared (Benfey, 1960; Benfey, Ledoux & Melville, 1960).

METHODS

Dogs anaesthetized with sodium pentobarbitone (30 mg/kg) were used in these experiments. The cord was transected between C1 and C2 in the spinal dogs. The contractile force of the exposed heart was recorded by a Cushny myocardiograph. Artificial respiration was given to the spinal and open-chest animals. The blood pressure was recorded from the carotid artery, and the following drugs were injected intravenously.

Phenoxybenzamine (Dibenzyline hydrochloride) and dibenamine hydrochloride were kindly supplied by Smith, Kline & French, Montreal. They were dissolved in 2 ml. of ethanol, and 8 ml. of physiological saline was added immediately before injection. Reserpine (Serpasil), guanethidine (Ismelin), and phentolamine (Rogitine hydrochloride), kindly supplied by Ciba Company, Montreal. Pentolinium tartrate (Ansolysen) and hexamethonium bromide, kindly supplied by Poulenc, Montreal. Dichloroisoproterenol (compound No. 20522), kindly supplied by Eli Lilly & Co., Indianapolis. Iproniazid (Marsilid), kindly supplied by Hoffmann-La Roche, Montreal. Adrenaline (Adrenalin chloride solution, Parke, Davis & Co.).

RESULTS

The intravenous injection of 20 mg/kg of phenoxybenzamine in a dog under pentobarbitone anaesthesia had the following effects (Fig. 1). There was an

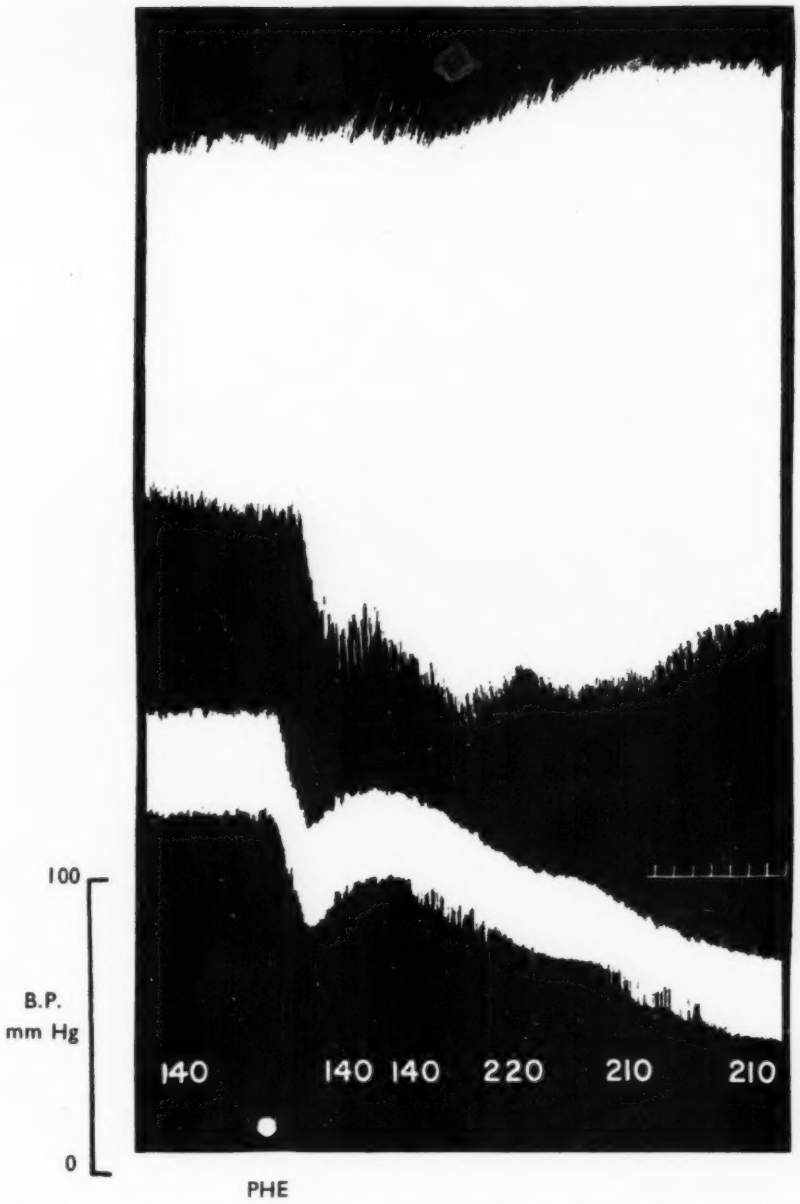

Fig. 1. Cardiac contractility and heart rate (upper record) and blood pressure (lower record) of a dog (9.8 kg) anaesthetized with pentobarbitone. Time in min. PHE: 20 mg/kg of phenoxybenzamine injected intravenously.

immediate increase in cardiac contractile force, there was a rise in heart rate 10 to 15 min later, and there was a fall in blood pressure. These inotropic, chronotropic and hypotensive effects were still present when the experiment was terminated after 3 hr.

Fig. 2 shows the actions of phenoxybenzamine in a dog treated with a ganglion-blocking drug. Ten mg/kg of pentolinium had little influence on cardiac contrac-

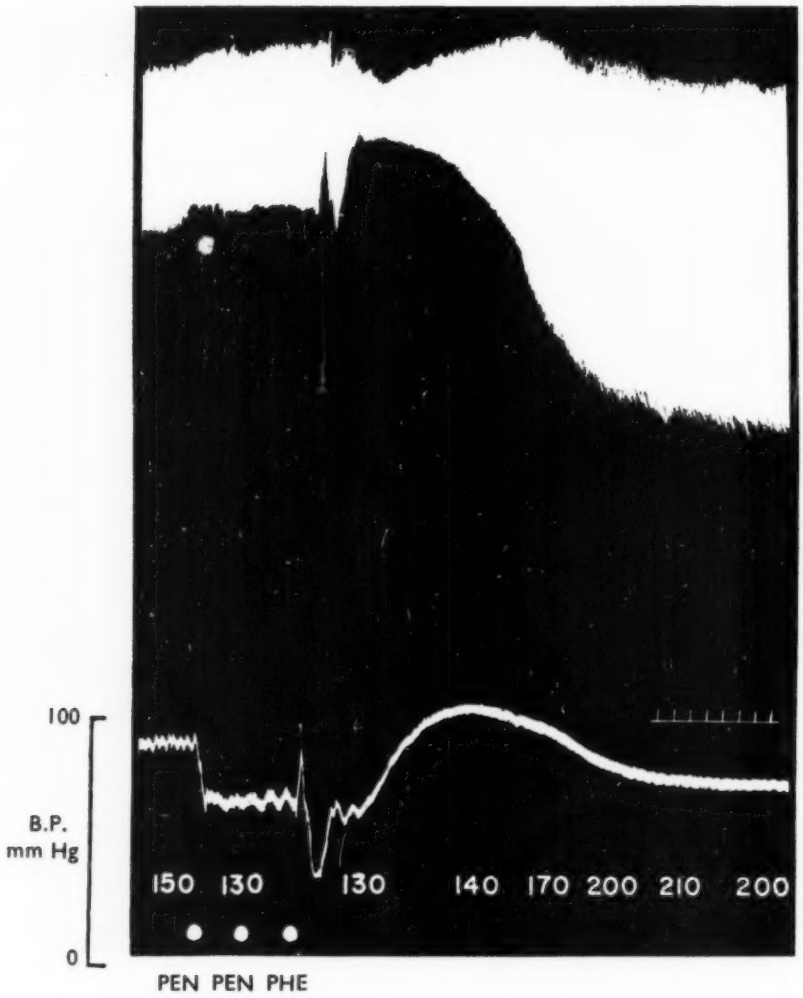

Fig. 2. Cardiac contractility and heart rate (upper record) and blood pressure (lower record) of a dog (7.2 kg) anaesthetized with pentobarbitone. Time in min. PEN: 5 mg/kg of pentolinium; PHE: 20 mg/kg of phenoxybenzamine injected intravenously.

tility, reduced the heart rate slightly, and lowered the blood pressure. Before it stimulated contractility and rate of the heart, phenoxybenzamine first depressed cardiac contractility for a period of approximately 10 min. While cardiac contractility was depressed the blood pressure rose to a peak and then fell when cardiac contractility began to increase.

The hypertensive action of phenoxybenzamine after ganglion-blocking drugs was variable and seemed to depend on the condition of the animal. When the blood pressure was normal, when few surgical procedures were carried out, and when the animal was kept warm, phenoxybenzamine could elevate the blood pressure to 200 mm Hg and keep it high for periods of more than 45 min. However, the rise was usually small and brief in an animal with a low blood pressure. Reserpine, a hypotensive agent with a transient hypertensive action in ganglionic blockade

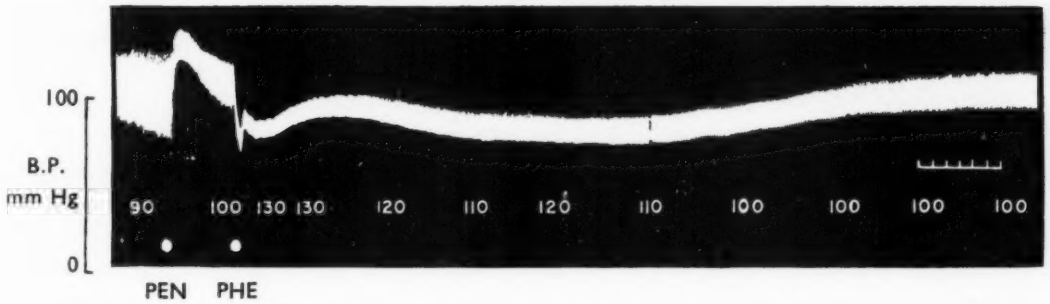

Fig. 3. Blood pressure and heart rate of a dog (8.1 kg) anaesthetized with pentobarbitone. Time in min. Reserpine (1 mg/kg) injected subcutaneously 24 hr before. PEN: 5 mg/kg of pentolinium ; PHE: 20 mg/kg of phenoxybenzamine injected intravenously.

(Maxwell *et al.*, 1957), was similarly found to have a poor hypertensive effect in dogs with a low blood pressure.

The dose of phenoxybenzamine required for the hypertensive effect (10 to 20 mg/kg) exceeded that sufficient for antisympathomimetic action (2 mg/kg). The time of injection was less than 1 min. Pentolinium was used in amount of 5 to 10 mg/kg and mostly given in two divided doses. Other ganglion-blocking drugs could be substituted for pentolinium and were all given in two divided doses, for example, tetraethylammonium (60 mg/kg), hexamethonium (30 mg/kg), pempidine (5 mg/kg). Atropine (4 mg/kg) had a similar action. The vagus nerves were cut only in the experiments in which atropine was given. Cutting the vagi did not enhance the hypertensive effect of phenoxybenzamine after ganglion-blocking drugs ; however, no experiments were carried out with atropine in animals whose vagus nerves were intact.

Pretreatment of the animals with reserpine or guanethidine prevented the inotropic, chronotropic and hypertensive actions of phenoxybenzamine in animals treated with ganglion-blocking agents ; this appears to indicate that they depend on the presence of endogenous adrenaline and noradrenaline. Fig. 3 shows that, in a dog pretreated subcutaneously with 1 mg/kg of reserpine 24 hr before, phenoxybenzamine given after pentolinium merely produced a slight and transient

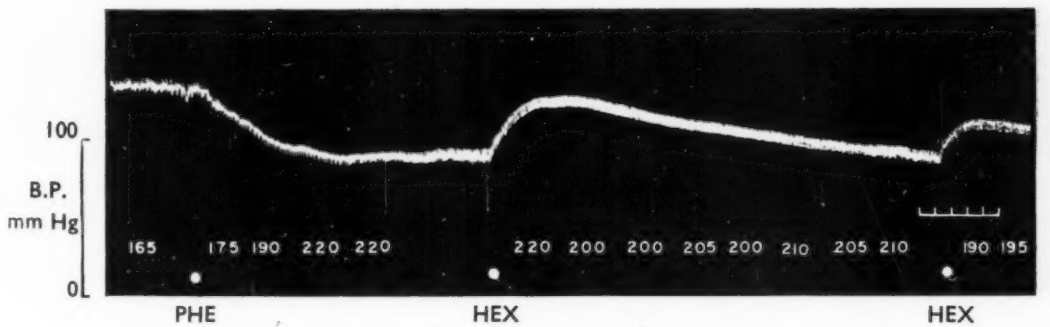

Fig. 4. Blood pressure and heart rate of a dog (11.6 kg) anaesthetized with pentobarbitone. Time in min. PHE: 20 mg/kg of phenoxybenzamine; HEX: 1 mg/kg of hexamethonium injected intravenously.

rise in heart rate and small fluctuations in blood pressure. The intravenous injection of 15 mg/kg of guanethidine 24 hr previously similarly prevented the stimulant actions of phenoxybenzamine after ganglion-blocking drugs. Cardiac contractility was depressed for a prolonged period and never increased, cardiac rate remained unchanged, and the blood pressure was rather stable. Pretreatment with guanethidine also abolished the inotropic, chronotropic and hypertensive actions of 0.5 to 1 mg/kg of reserpine given intravenously after ganglion-blocking drugs.

Ganglion-blocking drugs led to a rise in blood pressure in unanaesthetized dogs treated with reserpine (Maxwell *et al.*, 1957). A similar effect is shown in Fig. 4.

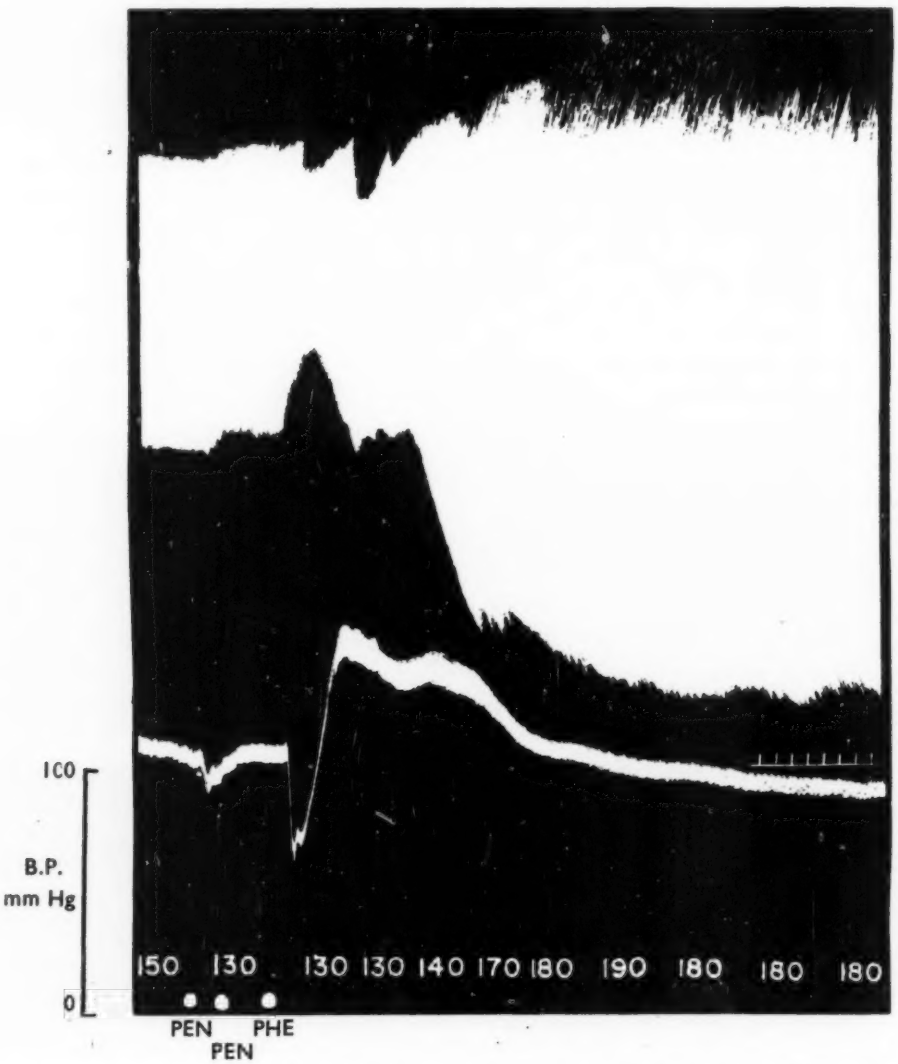

Fig. 5. Cardiac contractility and heart rate (upper record) and blood pressure (lower record) of a dog (9.2 kg) anaesthetized with pentobarbitone. Time in min. Phenoxybenzamine (20 mg/kg) injected intravenously 24 hr before. PEN: 2.5 mg/kg of pentolinium; PHE: 20 mg/kg of phenoxybenzamine injected intravenously.

One mg/ml. of hexamethonium produced a rise in blood pressure in an animal treated with 20 mg/kg of phenoxybenzamine.

Cocaine can potentiate actions of adrenaline and noradrenaline. The intravenous injection of 5 mg/kg of cocaine in ganglionic blockade greatly potentiated the hypertensive action of 0.5 to 1 mg/kg of reserpine injected intravenously 10 to 20 min later. Cocaine prevented the initial negative inotropic action of phenoxybenzamine in ganglionic blockade, but there was a poor hypertensive effect. The monoamine oxidase inhibitor iproniazid (20 mg/kg given intravenously 1 hr before) did not prevent the negative inotropic effect of phenoxybenzamine, and there was no rise in blood pressure in ganglionic blockade.

Reserpine elevates the blood pressure of a spinal dog (Maxwell *et al.*, 1957); phenoxybenzamine, however, caused a prolonged fall. An attempt was made to obtain a blood pressure rise with phenoxybenzamine in spinal dogs in ganglionic blockade by infusing small amounts of noradrenaline (10 to 50 ng/kg/min). However, there was no success although the blood pressure could be kept fairly high.

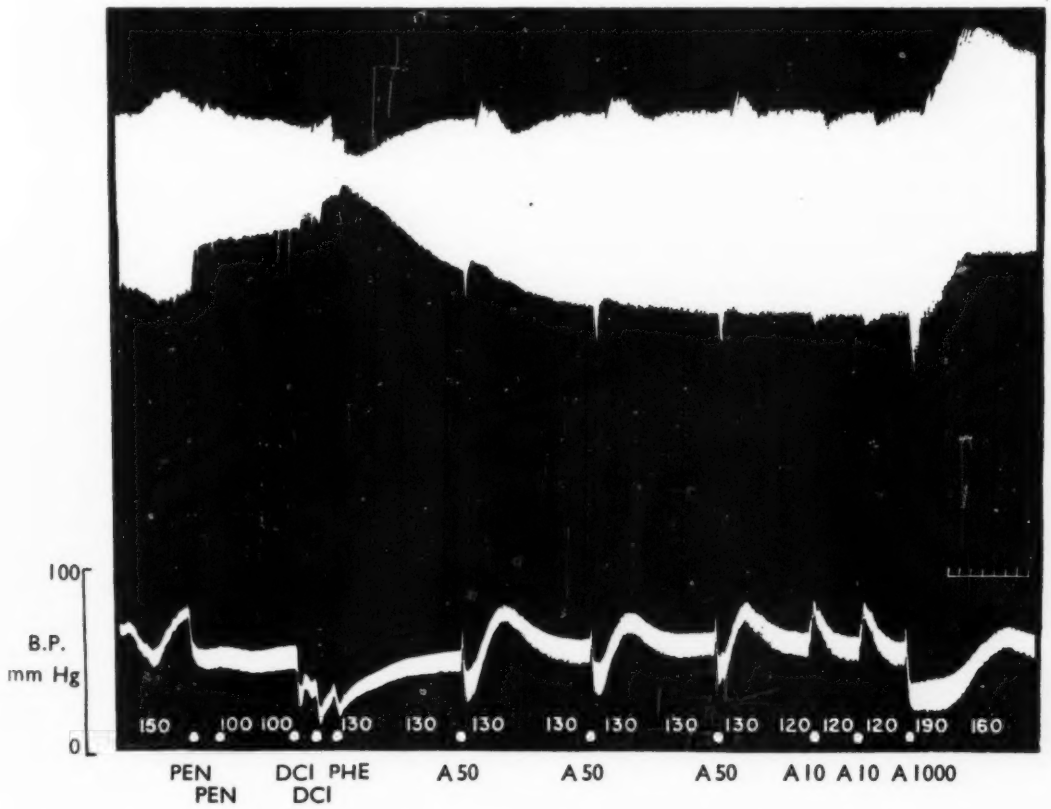

Fig. 6. Cardiac contractility and heart rate (upper record) and blood pressure (lower record) of a dog (7.2 kg) anaesthetized with pentobarbitone. Time in min. Phenoxybenzamine (20 mg/kg) injected intravenously 24 hr before. PEN: 2.5 mg/kg of pentolinium; DCI: 2.5 mg of dichloroisoproterenol; PHE: 20 mg/kg of phenoxybenzamine; A 50: 50 μg/kg of adrenaline; A 10: 10 μg/kg of adrenaline; A 1000: 1 mg/kg of adrenaline injected intravenously.

When phenoxybenzamine was injected in anaesthetized dogs after ganglion-blocking drugs during an infusion of adrenaline or noradrenaline (1 μg/kg/min), there was an immediate and deep fall in blood pressure. It appears that following phenoxybenzamine greatly increased amounts of circulating adrenaline and noradrenaline act strongly on the vasodilator receptors.

Antisympathomimetic drugs abolish the hypertensive action of reserpine (Maxwell *et al.*, 1957); however, pretreatment with phenoxybenzamine (20 mg/kg given intravenously 24 hr before) did not interfere with the typical actions of another dose of the drug after pentolinium (Fig. 5). This was surprising in view of the fact that phenoxybenzamine appeared to exert its hypertensive action through endogenous adrenaline and noradrenaline, but it was not possible to obtain a rise in blood pressure with injected adrenaline or noradrenaline 24 hr after phenoxybenzamine.

However, Fig. 6 shows that when a dog treated with 40 mg/kg of phenoxybenzamine in a period of 24 hr was given dichloroisoproterenol which interferes with the action of adrenaline on the heart and on vasodilator receptors, a small dose of adrenaline (10 μg/kg) could produce a rise in blood pressure, while a large dose (1 mg/kg) caused a blood pressure fall and an intermediate dose (50 μg/kg) had a mixed effect. The hypertensive effect of the small dose of adrenaline may have been due to an increase in cardiac output; however, little, if any, increase in cardiac contractility and no change in heart rate were demonstrable.

Dichloroisoproterenol could not be used satisfactorily to demonstrate sympathomimetic actions of phenoxybenzamine on the heart by blocking them because, although interfering with the effects of injected adrenaline, it strongly stimulated the contractile force and rate of the heart after ganglion-blocking drugs for a prolonged period of time. But Fig. 6 shows that when phenoxybenzamine was injected before the dichloroisoproterenol effects developed, there was only a small rise in cardiac contractility and rate, and adrenaline could further increase cardiac contractile force. In the absence of dichloroisoproterenol phenoxybenzamine generally stimulated cardiac contractility to such an extent that adrenaline could not further increase it. Dichloroisoproterenol also appeared to stimulate vasodilator receptors which prevented the blood pressure rise after phenoxybenzamine.

Certain other antisympathomimetic drugs did not have a hypertensive action following ganglion-blocking drugs. Twenty to 50 mg/kg of dibenamine, which is closely related to phenoxybenzamine, lowered the blood pressure and depressed cardiac contractile force for a long period of time. Five mg/kg of phentolamine depressed the heart so much that several injections of adrenaline were needed to re-establish its function.

DISCUSSION

The results suggest that the cardiovascular actions of phenoxybenzamine are probably mediated by adrenaline and noradrenaline. There was little change in cardiac contractility and rate and in blood pressure when pretreatment with reserpine or guanethidine had greatly reduced the amount of available adrenaline and noradrenaline. However, there was a fall in blood pressure when phenoxybenzamine was injected without pretreatment, and this was very marked in the presence of

infused adrenaline and noradrenaline. The increase in the plasma concentration and urinary excretion of adrenaline and noradrenaline caused by phenoxybenzamine (Millar *et al.*, 1959 ; Benfey *et al.*, 1959a and b) seems to contribute to the fall in blood pressure.

No rise in urinary catecholamine excretion was previously detected when phenoxybenzamine was injected during an infusion of hexamethonium or when the blood pressure fall was prevented by repeated injections of vasopressin. These results support the suggestion of Brown & Gillespie (1957) that phenoxybenzamine interferes with the inactivation of endogenous noradrenaline. An indirect action of phenoxybenzamine has also been reported by Hukovic (1959), who found an increased response of the isolated rabbit atrium to sympathetic stimulation.

The fact that phenoxybenzamine stimulated the heart in ganglionic blockade when sympathetic tone was greatly reduced may, however, indicate a direct action of the drug, that is, a release of adrenaline and noradrenaline in a manner similar to that of reserpine, although the methods of measuring urinary catecholamine output did not detect it. However, there are differences in the actions of the two drugs since a single dose of reserpine could prevent the cardiovascular effects of phenoxybenzamine and a single dose of phenoxybenzamine could not.

The blood pressure rise appears to be possible because the amount of circulating adrenaline and noradrenaline is low in ganglionic blockade. When signs of cardiac stimulation indicated an increase in circulating catecholamine, the blood pressure rise turned into a fall. The mechanism of the blood pressure rise following phenoxybenzamine is obscure. The rise in blood pressure was always followed by signs of cardiac stimulation. It is not likely that an increase in cardiac output produced the blood pressure rise because while it occurred cardiac contractility was depressed and the rate not changed. But sympathomimetic agents have not been reported to constrict blood vessels 24 hr after a full dose of phenoxybenzamine. It may be suggested that there is a difference between exogenous and endogenous catecholamine. When small amounts of adrenaline and noradrenaline are released in the body they may elevate the blood pressure by vasoconstriction in the presence of phenoxybenzamine and ganglion-blocking drugs. It has repeatedly been observed that ganglion-blocking drugs and atropine potentiate hypertensive actions of noradrenaline (Haas & Goldblatt, 1959). In the presence of phenoxybenzamine the greater amounts of adrenaline and noradrenaline released by reserpine act predominantly on vasodilator receptors.

It is, however, not necessary to assume that phenoxybenzamine releases adrenaline and noradrenaline. The drug may interfere with the inactivation of the transmitter substance of the sympathetic fibres and, in the presence of ganglion-blocking drugs or atropine, potentiate its action on the vasoconstrictor receptors instead of exerting its usual antisympathomimetic action.

A transient depressant effect on cardiac contractility became evident when phenoxybenzamine was given in ganglionic blockade. The related drug dibenamine had a very prolonged depressant action and never stimulated cardiac contractile force in ganglionic blockade. Dibenamine has been reported to have a negative inotropic action upon the heart-lung preparation of the dog and to diminish the rate of the isolated rabbit auricle (Acheson, Farah & French, 1949). Phentolamine

depressed the heart very strongly in ganglionic blockade. The drug was found to have a positive chronotropic action in dogs and cats; however, this was absent in the isolated heart (Gross, Tripod & Meier, 1951). Piperoxane had a quinidine-like action on the isolated rabbit auricle (Dawes, 1946). Thus, although previous studies in cats have shown that all of these antisympathomimetic agents increase the urinary output of adrenaline and noradrenaline in a manner similar to that of phenoxybenzamine (Benfey *et al.*, 1959b), it seems that strong depressant effects prevent a sympathomimetic stimulation of the heart.

Note added in proof

It has been found that in dogs subjected to transection of the cervical spinal cord and vagotomy phenoxybenzamine stimulated cardiac rate and contractility which were further increased by injecting reserpine an hour later; it has been concluded that phenoxybenzamine releases small amounts of catecholamines; and it has been suggested that the blockade of the adrenergic receptor involves both an impaired inactivation and a release of the sympathetic transmitter substance (Benfey & Varma, *Fed. Proc.*, in the press).

This work was supported by a grant from the National Research Council of Canada.

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THE EFFECT OF HEXAMETHONIUM ON THE CAROTID CHEMORECEPTOR RESPONSE TO NICOTINE AND CYANIDE

BY

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(*Received February 5*, 1960)

The literature concerning the effects of ganglionic blocking agents on the chemoreceptors is reviewed. Hexamethonium blocks the respiratory response to intracarotid injections of small doses of nicotine in dogs anaesthetized with chloralose, but it does not block the response to sodium cyanide.

There has been considerable controversy about the effects of hexamethonium and other ganglionic blocking agents on the chemoreceptor responses of the carotid and aortic bodies to various stimuli (see Table 1). In the course of our investigations of the actions of sympathomimetic amines on the carotid chemoreceptors (Byck & Pelikan, 1960), we have had occasion to use hexamethonium as a pharmacological tool in attempting to define the mode of action of certain drugs including that of sodium cyanide and nicotine. The following experiments were designed to resolve the controversy regarding hexamethonium chemoreceptor block.

METHODS

Thirteen dogs weighing from 7.4 to 14.2 kg were anaesthetized by intravenous injection of a 10% solution of chloralose in polyethylene glycol 200 (80 mg/kg) 1 hr after subcutaneous injection of morphine sulphate (1 mg/kg).

Cannulae were inserted into the trachea, a femoral artery, a femoral vein, and both superior thyroid arteries (the latter directed toward the common carotid artery). Since dogs given hexamethonium intravenously maintain a constant blood level of the drug after nephrectomy (Monge, Corcoran, DelGreco & Page, 1954), the renal artery and vein were ligated bilaterally before each experiment. The procedure was carried out through a midline abdominal incision and the wound was closed with sutures.

Respiration was recorded quantitatively by a modification of a bag-box system which separates inspired and expired gas (Donald & Christie, 1949). The animal inspired through a valve from a thin-walled, 50-litre meteorological balloon which was enclosed in an air-tight 60-gallon metal drum; this provided an inexpensive bag-box system which required filling infrequently. The animal expired air into the space around the bag through a valve. In the original method, Donald & Christie measured tidal vol. by recording the excursions of a spirometer connected to the inside of the box. In these experiments a sensitive strain gauge was substituted for the spirometer and the pressure fluctuations during inspiration and expira-

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tion were related to vol. of gas breathed. This method separates inspired and expired gas so that gases of different compositions may be breathed, but still permits a quantitative measure of tidal vol. The response time of this system was found to be less than 0.1 sec and the accuracy of measurement of a single breath to be approximately 2%. The tidal vol. of a single breath or respiratory min vol. over a period of time could thus be determined with ease. Arterial blood pressure was recorded from a femoral artery. Chemoreceptor stimulant drugs were injected in slightly greater than threshold concentrations into the common carotid blood stream via the cannulae in the superior thyroid arteries; 0.1 ml. of drug was flushed in with 1.0 ml. of 0.9% NaCl. Hexamethonium was given intravenously (50 to 200 mg total dose). The exact time of injection was marked on the record by a micro-switch triggered by the injection syringe. Injections were made into alternate arteries, allowing a total time lapse of at least 15 min between injections into any one artery. All intracarotid doses are total amounts.

In order to demonstrate that the respiratory response was due to chemoreceptor stimulation, all drugs were tested after section of the sinus nerve or infiltration of the area of the carotid bifurcation with 2% lignocaine.

Control injections. A control injection of 0.9% NaCl was given between all drug injections in exactly the same manner as the drugs; this never produced a significant response. Intravenous injections of polyethylene glycol 200 in 2 dogs anaesthetized with 1% chloralose dissolved in warm saline did not diminish the respiratory response to chemoreceptor stimulants.

All drugs were prepared as fresh stock solutions in 0.9% NaCl. Nicotine was used as the base and hexamethonium as the chloride.

Polyglycol E-200 was provided through the courtesy of the Dow Chemical Company, Midland, Michigan. The chloralose used in these experiments was "Pur alpha chloralose," Kuhlmann Produits R.A.L., Paris.

RESULTS

Measurement of records. The usual response to intracarotid injection of small doses of chemoreceptor stimulants was a single deep breath followed by an almost immediate return to normal respiration (Figs. 1 and 3). Larger doses caused an intense hyperpnoea of variable duration. The response to small doses was related to the dose given; in a series of 10 dogs in which 64 doses of nicotine (0.2 to 1.0 μg) were given, an increase in dose resulted in an increased response in 90% of the tests. Since the dose-response curve was not linear over a larger range of doses, we used a minimal effective dose as a standard of comparison. The ED30 was chosen as a reliable estimate because it was found that this response was comfortably above the normal variations in respiration.

The maximal effect on tidal vol. was limited by the maximal inspiratory capacity of the dog. Supramaximal doses did not produce consistent maximal inspiratory efforts in the first 5 sec and so the data could not be plotted as a difference from a known maximal response. Plotting the responses in this way (using the largest breath the dog made with any dose as a maximum) did not reduce the variability seen in the responses to a single dose given at different times in the experiment. Transformation of the data in other ways such as dose against absolute increase in tidal vol. or dose against vol. of maximal breath in the response similarly did not reduce the variability. Since diminished control tidal vol. seemed to be a function of depth of anaesthesia and was associated with diminished responsiveness of the animal, percentage increase of tidal vol. above control tidal vol. was used as the measure of response.

As a guide to the significance of the blocking effects, the ED30 of nicotine (10 dogs) was 0.28 μg. From this figure, shown graphically on a log. scale in Fig. 2,

an ED30 which is greater by a statistically significant margin can be estimated. A significant block was considered to have been demonstrated when the ED30 after ganglionic blockade was more than 3 standard deviations from the control ED30. Equivalent figures were calculated for sodium cyanide and established as criteria for block.

Nicotine. In low doses there was a clear relationship between the dose used and the increase in ventilation. This response was abolished by section or block of Hering's nerve.

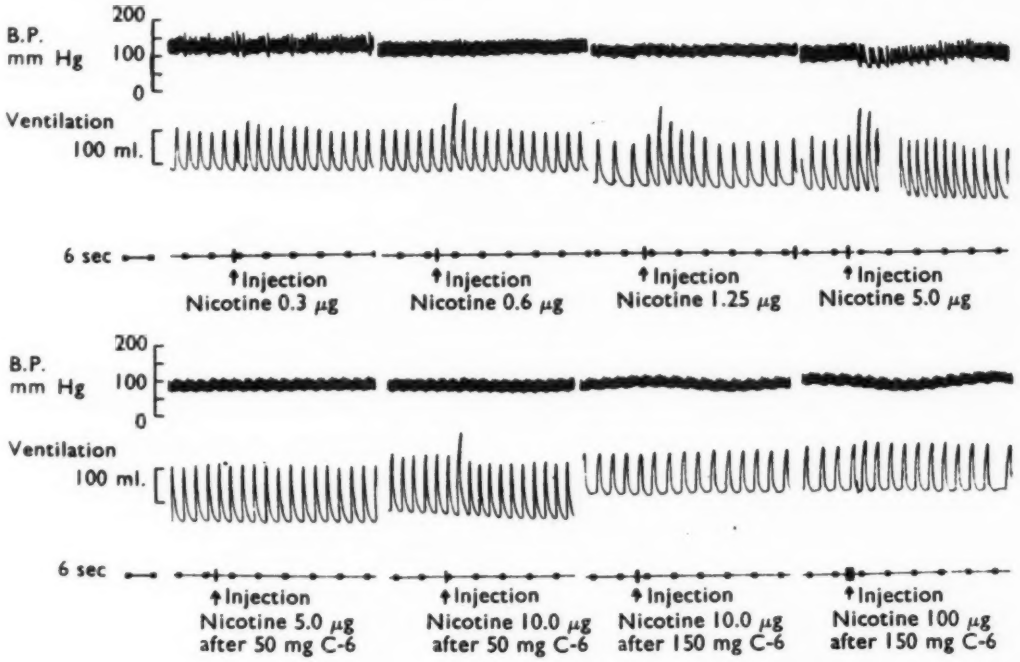

Fig. 1. Blood pressure and respiration responses to graded intracarotid doses of nicotine in a dog under chloralose anaesthesia before and after hexamethonium (C-6) given intravenously.

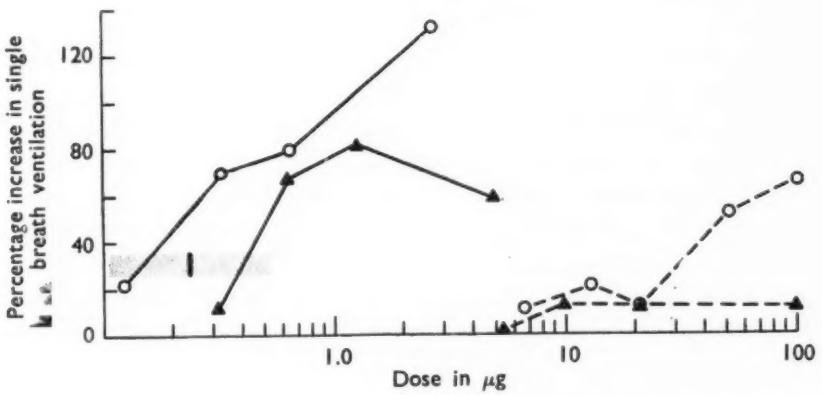

Fig. 2. Dose-effect (respiration) curves for graded intracarotid doses of nicotine in two experiments. Shaded area shows one standard deviation on each side of the mean ED30 for 10 dogs. Expt. 1: before ▲——▲, after ▲ – – ▲. Expt. 2: before O——O, after O – – O (50 mg C-6).

In Fig. 1 responses to nicotine before and after administration of hexamethonium are illustrated. There is a blockade of previously effective small doses. Larger doses of nicotine still caused a response. In all dogs tested 50 mg of hexamethonium abolished the response of intracarotid doses of nicotine up to 6.25 μg. At doses between 6.25 and 100 μg, the hexamethonium block was not consistently obtained. The response to 100 μg of nicotine could not be blocked even when an almost lethal dose of hexamethonium was administered; it was therefore concluded that hexamethonium causes a competitive block of the chemoreceptor response to nicotine. The shift in the dose-effect curve in 2 experiments is shown in Fig. 2.

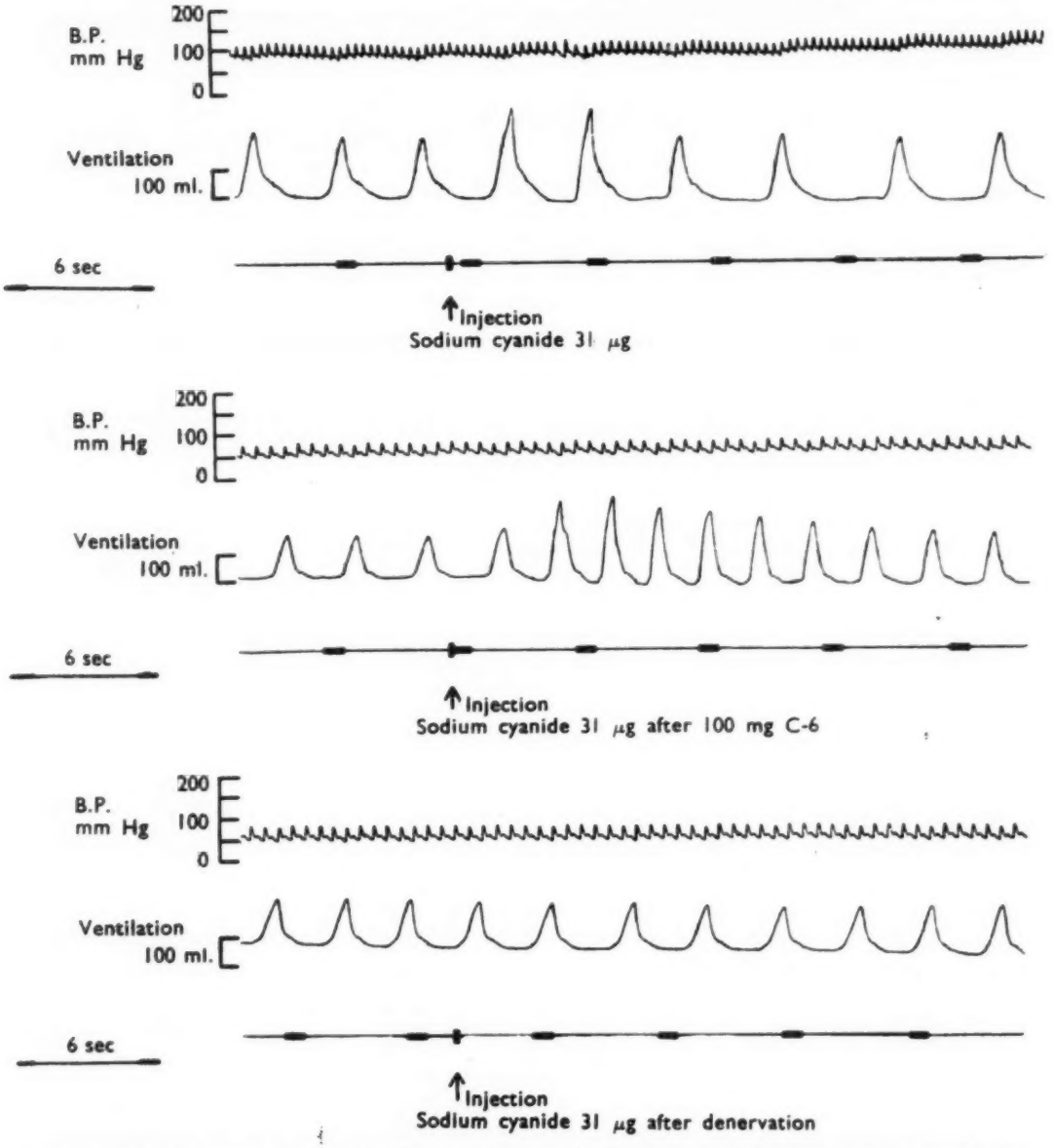

Fig. 3. Blood pressure and respiration responses to intracarotid injections of sodium cyanide in a dog under chloralose anaesthesia before and after hexamethonium and denervation.

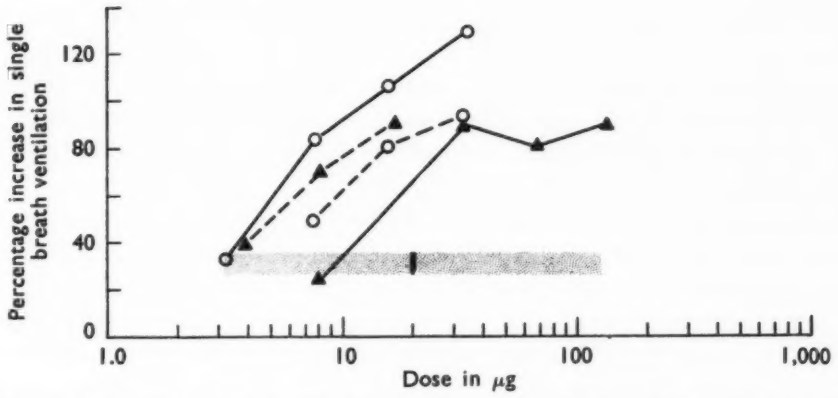

Fig. 4. Dose-effect (respiration) curves for graded intracarotid doses of sodium cyanide in 2 experiments. Shaded area shows one standard deviation on each side of the mean ED30 for 10 dogs. Expt. 1: before ▲——▲, after ▲ – – ▲. Expt. 2: before O——O, after O – – O (50 mg C-6).

Sodium cyanide. Again a clear relation was found between the dose and the increase in response over the dose range used. There was no evidence of diminution of response to cyanide after any dose of hexamethonium (Fig. 3). In some animals there appeared to be a potentiation of the response to cyanide after hexamethonium. This potentiation might well be due to the increased excitability of the chemoreceptors secondary to a decrease in blood pressure (Floyd & Neil, 1952). It also might be analogous to the increased duration of response to cyanide found in studies recording nerve impulses in the sinus nerve of the cat (Dontas & Nickerson, 1956). The shift in the dose-effect curve is shown in Fig. 4.

DISCUSSION

We believe that the controversy regarding the effect of hexamethonium on the carotid chemoreceptors is due to disagreement on three points:

1. What is the proper criterion for chemoreceptor stimulation? Are changes in respiration and blood pressure too non-specific to allow conclusions about the effects of drugs on the chemoreceptors?

2. Can a competitive block be found if supramaximal doses of stimulant drugs are used and, conversely, is there any meaning to a block found after massive intra-arterial doses of blocking agents?

3. Must a response be inhibited almost completely to demonstrate block (Heymans, Delaunois, Martini & Janssen, 1953), or is a significant shift of the dose-response curve sufficient evidence of inhibition or block?

First let us discuss the method of recording and measurement. Recording of respiratory parameters is a direct measurement of the end result of chemoreceptor action. This is desirable because it provides a measure of the total respiratory effect of the injected drug. If one could eliminate the other variables which might affect respiration, this would be the ideal method of evaluating chemoreceptor stimulant activity. To utilize the advantages of the respiratory measurement, one must be able to minimize interfering factors, detect subtle differences in volume or

rate, and finally, make a quantitative estimate of the respiratory response in a significant way.

A quantitative estimate of the respiratory response is possible using several different parameters. The use of the bag-box system allows calculations of tidal volume of single breaths as well as respiratory minute volume over any selected interval of time.

Recordings from the sinus nerve can certainly detect changes in afferent activity, but there may be uncertainty about the receptors stimulated, and whether the impulses are directed to the respiratory centre or to other regions. The method lends itself to assay, although this possibility has not yet been fully explored.

The second point of contention has been dosages, both of the chemoreceptor stimulants and the blocking agents. If one is to obtain significant results, one should use doses of drugs that could conceivably be blocked by competitive agents such as hexamethonium. The doses used by Heymans *et al.* (1953), which produced a marked hyperpnoea and where no dose-effect relationship could be seen, are clearly supramaximal. An equal imbalance is seen in the use of large intra-arterial doses of blocking agents. As Heymans & Neil (1958) comment, " We are somewhat dubious about the effects of huge amounts of these drugs given locally into the carotid circulation. We feel that the *p*H of the solutions used and the bromide or iodide ion of the compounds may be complicating the responses seen in these conditions."

The final element of design is the criterion of block. Considering the mode of action of hexamethonium, one would not expect a complete block of any compound *a priori* but, rather, would expect a significant shift of the dose-effect curve or a significant diminution of the response to individual doses. Heymans *et al.* (1953) feel that complete inhibition of the respiratory response is necessary to demonstrate blockade.

In our experiments we have attempted to meet all of these objections as follows. (1) A direct and quantitative measure of response which fits a dose-effect relationship was used (Byck, 1957). (2) The doses of both chemoreceptor stimulant drugs and blocking agents were varied. The doses of the chemoreceptor stimulants varied from just threshold (for example, 0.3 μg nicotine) to supramaximal (for example, 100 μg nicotine). The doses of the blocking agent used, although high, were not excessive and were given by the intravenous rather than the intra-arterial route. (3) The presence of a block was demonstrated by the use of dose-effect curves as well as by the illustration of the inhibited response.

Different preparations, animals and anaesthetics have been used in the various investigations cited in Table 1. Since our results are diametrically opposed to two of these, namely, Heymans *et al.* (1953) and Boelaert (1948), it is necessary to examine their experiments in some detail. Both authors measured the respiratory responses of dogs under chloralose anaesthesia. Heymans *et al.* (1953) used both hexamethonium and tetraethylammonium. They used respiratory min vol. as a measure of effect, and, although they emphasize the importance of the dose-effect relationship as a basis for drawing conclusions, they gave no data on varying responses to varying doses. Further, in their tracing in this paper on p. 215 there is two to three times the respiratory response to the same dose of lobeline and acetylcholine

TABLE I

THE EFFECT OF GANGLIONIC BLOCKING AGENTS ON CHEMORECEPTOR RESPONSES

Authors	Animal	Preparation and recording	Anaesthesia	Blocking agent and dose	Route of administration of blocking agent
A. Block of nicotine-like drugs and no block of cyanide or anoxia					
Dontas & Nickerson (1956)	Cat	Record from sinus nerve	Chloralose	Hexamethonium 1.0 mg total dose	Close intra-arterial injection
Douglas (1952)	Cat	Crossed circulation, record of respiration	Pentobarbitone sodium	Hexamethonium 15 mg/kg	Intravenous
Moe *et al.* (1948)	Dog	Record of respiration	Thiopentone + barbitone	Tetraethylammonium 20 to 40 mg/kg/hr	Continuous intravenous infusion
This paper	Dog	Record of respiration	Chloralose	Hexamethonium 50 to 200 mg (5 to 20 mg/kg)	Intravenous
B. Block or decreased response to both nicotine-like drugs and cyanide or anoxia					
Landgren *et al.* (1952)	Cat	Record from sinus nerve	Chloralose Urethane	Tetraethylammonium 100 mg	Close intra-arterial injection
				200 mg	Intravenous
Gollwitzer-Meier (1953)	Cat	Record from sinus nerve	Chloralose	Hexamethonium 1.0 mg total dose	Close intra-arterial injection
C. Block of neither nicotine-like drugs nor cyanide or anoxia					
Heymans *et al.* (1953)	Dog	Record of respiration	Chloralose	Hexamethonium 2 to 5 mg	Locally into conjunctival sac
				Tetraethylammonium 10 mg/kg	Intravenous
Boelaert (1948) (As quoted by Heymans and Neil, 1958)	Dog	Not stated	Chloralose	Tetraethylammonium—dose not stated	Not stated

before hexamethonium as after hexamethonium, whereas the response to cyanide did not change appreciably. Further, because of the relatively short duration of action of ganglionic blocking agents, it is necessary to have some method of keeping constant blood levels of the drugs in order to compare blocking activity. Moe, Capo & Peralta (1948) used a continuous intravenous infusion of tetraethylammonium. In the present experiments the dogs were "nephrectomized." Heymans *et al.* (1953), however, had no method of keeping a constant level of blocking agent in their experiments, and they do not record or comment on the duration of blockade achieved with a single injection technique; therefore, even a greater degree of block may have been present at the time of highest tissue concentration.

We cannot comment on the work of Boelaert (1948) because of the lack of specific information in the paper as published.

The experiments of Landgren, Liljestrand & Zotterman (1952) and Gollwitzer-Meier & Witzleb (1953) are open to the criticism previously quoted from Heymans & Neil (1958), that very concentrated intra-arterial doses of blocking agents may have non-specific actions. Indeed, when Landgren *et al.* (1952) used intravenous doses of blocking agents, they too demonstrated a diminished response to nicotine-like but not to anoxic stimulation.

I would like to thank Dr. Julius H. Comroe, Jr., for his invaluable advice and assistance in the preparation of this manuscript. This investigation was supported in part by a grant from the United States Public Health Service to the University of California (H-4029).

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Brit. J. Pharmacol. (1961), **16**, 23–31.

THE EFFECT OF MORPHINE ON THE ADRENERGIC NERVES OF THE ISOLATED GUINEA-PIG JEJUNUM

BY

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(*Received May* 30, 1960)

Nerves accompanying the mesenteric blood-vessels of the isolated guinea-pig jejunum were stimulated and changes in the longitudinal muscle coat were recorded. Stimulation of the nerves led to a rapid relaxation if the intestine was contracted by a previous administration of histamine. The relaxation was inhibited by morphine and partially restored by nalorphine. Bretylium and atropine also inhibited the relaxation and cocaine increased it somewhat. Morphine, atropine, and bretylium had no significant effect on the depressant action of noradrenaline on histamine contractions, whereas cocaine slightly enhanced the action of noradrenaline. Morphine, but not the other drugs, prevented the inhibitory action of dopamine.

Morphine and other narcotics inhibit contractions due to postganglionic cholinergic stimulation, by reducing the release of acetylcholine from nerve endings in the isolated guinea-pig intestine (Paton, 1957). Morphine decreases the cardiac slowing produced by vagal stimulation in the rabbit, rat, and to some extent in the cat (Kosterlitz & Taylor, 1959). Interference by morphine with the release of noradrenaline from the adrenergic nerve endings of the cat nictitating membrane has also been reported by Trendelenburg (1957). This paper describes the action of morphine on the adrenergic nerves of the isolated guinea-pig jejunum. In order to obtain a fuller understanding of the mechanism of action of morphine on this relatively simple preparation, other agents known to affect autonomic nerves were also included in this study.

METHODS

For stimulating the nerves leading to the intestine a preparation similar to that described by Finkleman (1930) for the rabbit gut was used. Freshly fed male guinea-pigs, weighing between 350 and 500 g, were killed by a blow on the head and 3 to 5 cm long pieces from the oral part of the jejunum were excised along with the attached mesentery. After washing the lumen of the intestine, the mesentery containing the artery supplying the gut was attached to shielded, silver, bipolar electrodes. The preparation including the electrodes was immersed in a 50 ml. bath, in such a way as not to interfere with the contraction or relaxation of the longitudinal muscle coat. Nerves accompanying the blood-vessels in the mesentery were stimulated by means of an Arthur H. Thomas square-wave stimulator, with impulses of 0.5 msec duration and with frequencies described in the section on results. There was no need for any further insulation of the electrodes from the bath fluid, as maximal relaxation from stimulation could be obtained by setting the output at 5 to 20 V. If the electrodes were immersed in the bath without the mesentery attached to them, or when the mesentery was tied between the electrodes and the intestine, electrical impulses that produced maximal relaxation before became ineffective. This showed that the impulses reached the intestine only through the mesenteric nerves.

Stimulation of the periarterial nerves of a quiescent piece of intestine led to a biphasic response, consisting of an initial brief contraction followed by a more prolonged relaxation, similar to that described by Munro (1953). In order to investigate how drugs affect the relaxation of the longitudinal muscle coat brought about by stimulating the mesenteric nerves, it was found necessary to apply the stimulus when the muscle was contracted by the addition to the bath of acetylcholine or histamine. Otherwise, the inhibition produced by depressant drugs would have precluded any further relaxation. In preference to acetylcholine, histamine was chosen to increase the tonus of the intestine, because the inhibition resulting from stimulation was greater in the case of histamine. Moreover, the use of histamine allowed the action of atropine to be observed. In order to maintain the tonus of the intestine at about constant level at the time of stimulation, histamine contractions had to be alternated with periods of rest. Without these rest periods the tonus of the intestine declined rapidly, in spite of the continued presence of histamine. For this reason the following procedure was adopted: every 4 min the intestine was exposed to 0.8 to 4×10^{-7} histamine which produced nearly maximal contraction. One min later, when the intestine had reached a constant level of contraction (that could be taken as a base-line for assessing the effect of nervous stimulation) the mesenteric nerves were stimulated for 5 or 15 sec. Following the relaxation resulting from stimulation, the intestine usually regained its pre-stimulation tonus by the time histamine was washed out of the bath, 2 min after its administration. The degree of relaxation produced by stimulation remained constant when this cycle of events was repeated many times. It was noted, however, that pieces from the more anal parts of the small intestine did not maintain a constant level of contraction for even 2 min, whereas the more oral parts (jejunum) responded to histamine with a steady contraction for this length of time. In addition, the jejunum proved to be more sensitive to the effects of morphine, and for these reasons it was used in all experiments described in this paper.

In the second part of the experiments the depressant effect of sympathomimetic amines on the contractions produced by histamine was measured. The intestine, suspended in a 10 ml. bath, was exposed to a constant amount of histamine (0.2 to 0.5 μg) every 3 min for 30 sec. The bath was then flushed for 20 sec. These operations were performed automatically by means of two Fisher electro-hosecocks connected to relays. The relays were activated by contacts made through perforations in the paper of a kymograph drum, revolving every 3 min.

Contractions of the longitudinal muscle coat were recorded by means of a light, isotonic, frontal lever, giving a magnification of 4:1. A Tyrode solution containing 8 g NaCl, 0.2 g KCl, 0.2 g $CaCl_2$, 0.09 g $MgSO_4$, 1 g $NaHCO_3$, and 1 g glucose per l. and aerated with a mixture of 95% oxygen and 5% carbon dioxide was employed throughout. All experiments, except those specifically mentioned, were performed at 37° C bath temperature. Morphine sulphate, bretylium tosylate, cocaine hydrochloride, atropine sulphate, hexamethonium chloride or pentolinium tartrate was dissolved in the Tyrode solution contained in the reservoir, while nalorphine hydrochloride, acetylcholine bromide, noradrenaline, dopamine (3-hydroxy-tyramine) hydrochloride, and ephedrine sulphate were added to the bath by means of syringes. Nalorphine was dissolved in Tyrode, acetylcholine in 0.9% NaCl, whereas noradrenaline, dopamine, or ephedrine was dissolved in distilled water containing 1 mg % ascorbic acid and injected in a constant volume of 0.1 ml. Histamine acid phosphate, dissolved in Tyrode solution, was administered with a syringe in experiments involving nervous stimulation, whereas in the experiment described in the second part, histamine solution was delivered into the bath from a small Marriotte bottle. All drug concentrations, with the exception of those of noradrenaline, hexamethonium, and pentolinium, refer to the salt form and are expressed in units of g/ml.

RESULTS

Stimulation of adrenergic nerves

Morphine. The relaxation resulting from mesenteric nerve stimulation of the jejunum, with a voltage about 50% above maximal, was reduced or abolished by 10^{-8} to 10^{-6} morphine. As shown in Fig. 1, replacing the bath fluid with one

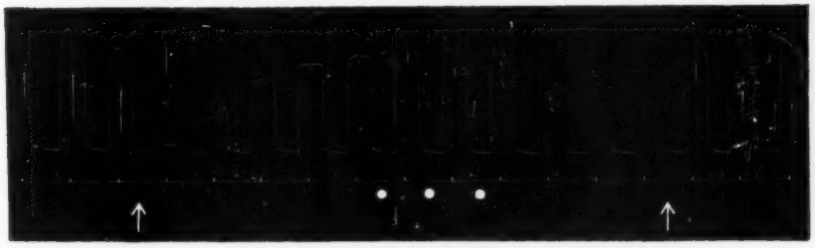

Fig. 1. Effect of morphine and nalorphine on the inhibition produced by mesenteric nerve stimulation. Every 4 min 20 μg histamine added, followed 1 min later by supramaximal stimulation with 20 pulses/sec for 5 sec. Between arrows 10^{-7} morphine in the bath fluid; at N 0.5 μg nalorphine added to the bath. Bath vol. 50 ml.

containing morphine, first considerably reduced the height of histamine contractions. At the same time, the effect of stimulation also decreased. Later, the histamine contractions returned to normal, whereas the relaxation due to stimulation was still diminished. Following the removal of morphine, the effectiveness of stimulation gradually returned. No evidence was found that morphine was more effective when submaximal voltage was employed or the frequency of stimulation was decreased.

Nalorphine. Nalorphine alone reduced the effect of adrenergic stimulation only slightly in a concentration of 10^{-6}, whereas smaller concentrations were without an effect. However, in the presence of morphine (10^{-7}) concentrations of nalorphine ranging from 2×10^{-9} to 2×10^{-7} increased the effect of adrenergic stimulation (Fig. 1). The antagonism of nalorphine to morphine was rather weak: it could be shown only when morphine did not fully inhibit the effect of stimulation. Even when nalorphine antagonized the action of morphine, it failed to restore fully the effectiveness of stimulation. Frequently, nalorphine increased the size of histamine contractions which were depressed by morphine. It was not possible to obtain quantitative data on the optimal concentration of nalorphine required to antagonize any given concentration of morphine because the action of morphine varied greatly from one preparation to the other. In various experiments, nalorphine was found to be an effective antagonist when present in a concentration of 1/50 to twice that of morphine.

Bretylium. This drug has been shown to reduce selectively the effect of adrenergic stimulation by preventing the release of noradrenaline from nerve endings (Boura & Green, 1959). It has been included in this study to ascertain the nature of the inhibition resulting from mesenteric nervous stimulation. Bretylium 10^{-6} progressively reduced the relaxation due to stimulation, until in about 30 min the inhibition became complete (Fig. 2). Washing out of the drug from the bath restored the relaxation only very slowly.

Atropine. The effect of atropine was tested because it was thought that by eliminating the cholinergic effect of stimulating a mixed nerve, it would increase the inhibition produced by the stimulation. Surprisingly, in even small concentrations (10^{-8}), atropine prevented the relaxation due to stimulation (Fig. 3). The

Fig. 2. Effect of bretylium on the inhibition due to mesenteric nerve stimulation. Every 4 min 20 μg histamine added, 1 min later supramaximal stimulation with 20 pulses/sec for 15 sec. At the beginning of (*b*) bretylium 10^{-6} added to the bath fluid. Bath vol. 50 ml.

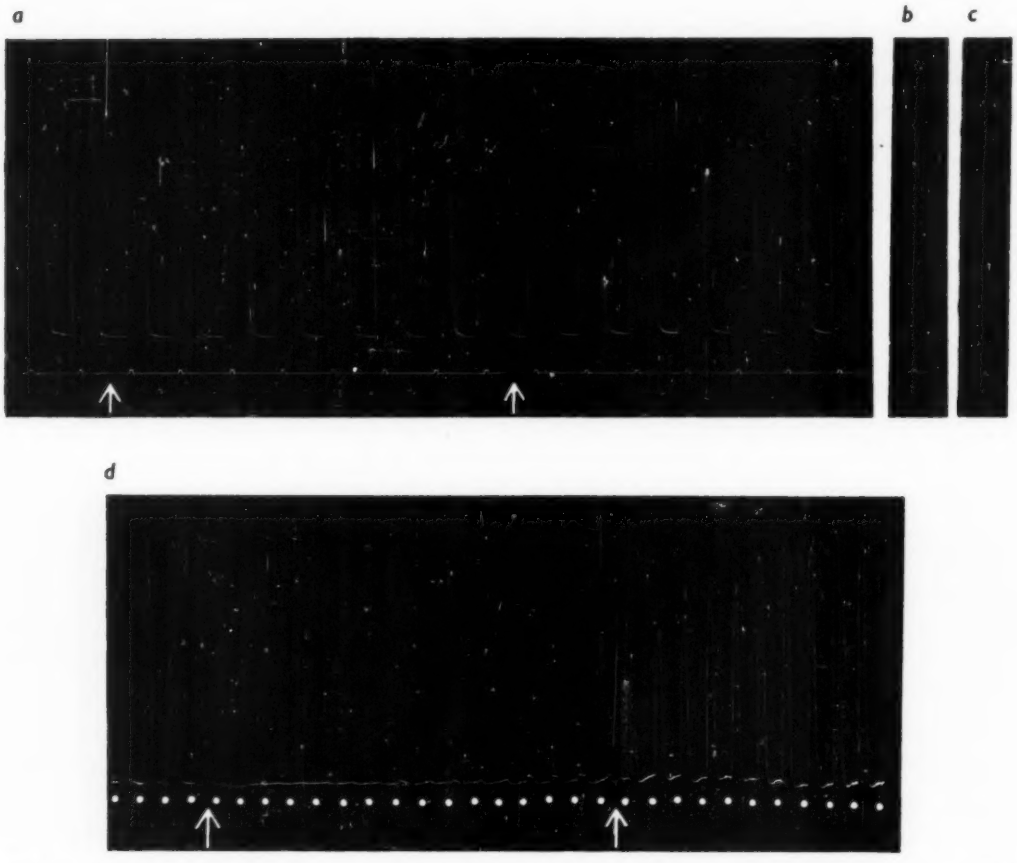

Fig. 3. (*a*) Effect of atropine on the inhibition due to mesenteric nerve stimulation. Every 4 min 10 μg histamine added, 1 min later supramaximal stimulation with 20 pulses/sec for 15 sec. Between arrows 10^{-8} atropine present in the bath fluid. (*b*) 32 min after *a*; (*c*) 16 min after *b*. (*d*) Effect of atropine on acetylcholine contractions. Every 2 min 2 μg acetylcholine added to the bath. Between arrows 10^{-8} atropine present in bath fluid. Bath vol. 50 ml.

inhibition was slow in onset and, once established, was slow to disappear after the drug has been removed from the bath. This course of events was entirely different from the antagonism of acetylcholine by atropine (Fig. 3*d*). Contractions caused by acetylcholine were immediately inhibited and returned to normal soon after atropine was washed out.

Cocaine and hexamethonium. Cocaine, in a concentration of 0.5 to 1×10^{-5}, increased the relaxation due to submaximal stimulation and prolonged somewhat the relaxation from supramaximal stimulation (Fig. 4). On the other hand, hexamethonium (1 to 2×10^{-5}) was without effect.

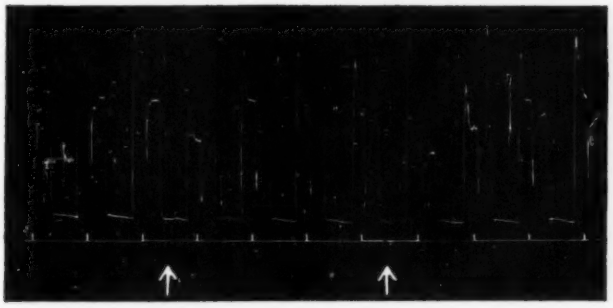

Fig. 4. Effect of cocaine on the inhibition produced by mesenteric nerve stimulation. Every 4 min 4 μg histamine added, followed 1 min later by supramaximal stimulation with 20 pulses/sec for 5 sec. Between arrows 0.5×10^{-5} cocaine present in bath fluid. Bath vol. 50 ml.

Action of sympathomimetic amines

Certain sympathomimetic amines act indirectly, through the release of noradrenaline from adrenergic nerve endings (Burn & Rand, 1958a). This observation afforded another method for assessing the action of morphine on the release of noradrenaline. In addition to noradrenaline, tyramine, dopamine, ephedrine and amphetamine were tested for their ability to inhibit histamine contractions. Of these tyramine and amphetamine stimulated the intestine and therefore had to be discarded. The other three amines had a purely depressant effect.

In order to measure the inhibitory action of the sympathomimetics it was necessary to obtain constant contractions to repeated submaximal doses of histamine. At 37° C, in the absence of any drug, the intestine showed considerable spontaneous activity and the responses to histamine varied a great deal. By adding 0.5×10^{-5} pentolinium to the bath fluid, however, the responses to histamine became more regular. Under these conditions exposing the intestine for 2 min to 2×10^{-8} noradrenaline or to 2×10^{-6} dopamine reduced the height of the subsequent histamine contraction by 10 to 70%, dopamine being somewhat more depressant than noradrenaline on the same piece of jejunum. Following the washing out of the sympathomimetic amine and the subsequent addition of histamine, the intestine often contracted, as shown in Fig. 5. As the experiment progressed, the intestine became more sensitive to the inhibitory effect of the sympathomimetics.

Introduction of 10^{-7} morphine into the bath fluid first greatly depressed the size of histamine contractions. After the response to histamine had become stabilized,

somewhat below the control level, dopamine inhibited only slightly the histamine contractions, whereas the depression produced by noradrenaline was affected much less by the presence of morphine (Fig. 5). Not only was the relative reduction

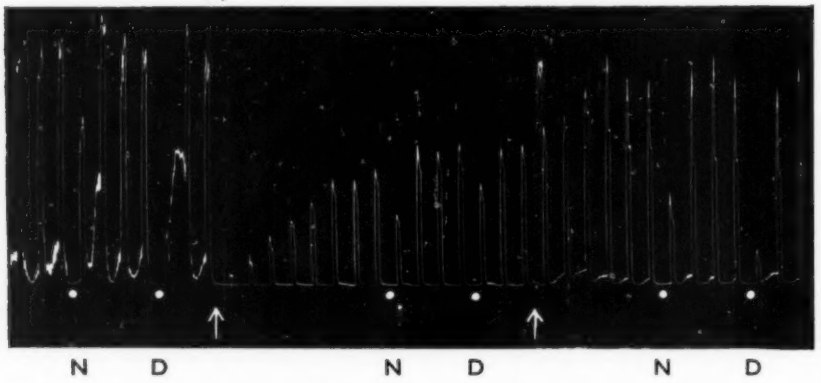

Fig. 5. Effect of morphine on the inhibitory action of noradrenaline and dopamine. Bath temperature 37° C. Every 3 min 0.25 μg histamine added. At N 0.2 μg noradrenaline, at D 20 μg dopamine added to bath. Between arrows 10^{-7} morphine present in bath fluid. Pentolinium 5×10^{-6} present throughout. Bath vol. 10 ml.

in the size of histamine contractions following dopamine less when morphine was present, but the absolute height of contractions after dopamine was larger than in the absence of morphine. After the removal of morphine from the bath, the inhibitory effect of dopamine was fully restored. When the bath temperature was kept at 28° C, the depressant action of dopamine greatly decreased. At 37° C 2×10^{-6} dopamine was more effective than 2×10^{-8} noradrenaline in reducing contractions to histamine. But at 28° C 2×10^{-6} dopamine did not produce any inhibition, and even twice that concentration was less effective than 2×10^{-8} noradrenaline (Fig. 8). Morphine did not affect the reduced response to dopamine at this lower temperature.

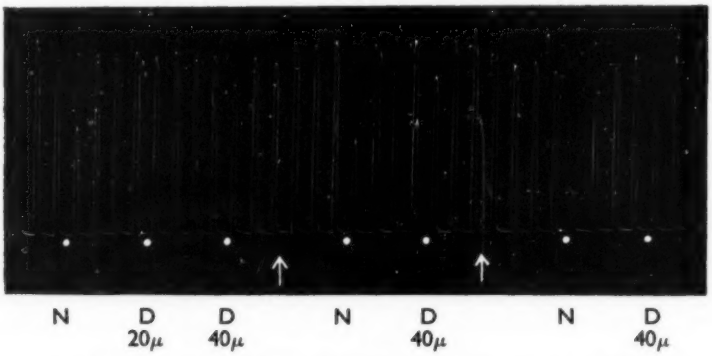

Fig. 6. Effect of morphine on the inhibitory action of noradrenaline and dopamine. Bath temperature 28° C. Every 3 min 0.25 μg histamine added. At N 0.2 μg noradrenaline, at D dopamine in amounts indicated added to bath fluid. Between arrows 10^{-7} morphine present in bath fluid. Pentolinium 5×10^{-6} present throughout. Bath vol. 10 ml.

At 37° C atropine (10^{-8}) did not have any significant effect on the inhibitory action of either dopamine or noradrenaline (Fig. 7). Cocaine 10^{-5} increased somewhat the action of noradrenaline, while leaving that of dopamine unaltered (Fig. 8). Bretylium 10^{-6} did not have any effect on the inhibition produced by any of the amines tested. In all the above experiments the action of ephedrine 10^{-5} was the same as that of noradrenaline.

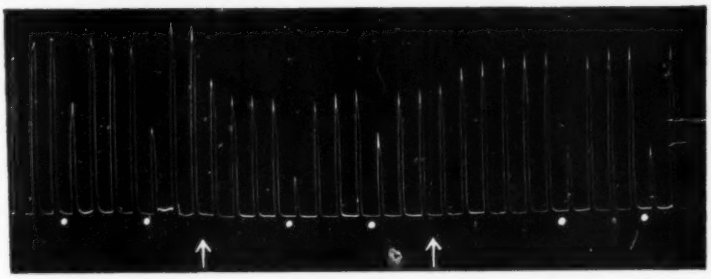

Fig. 7. Effect of atropine on the inhibitory action of noradrenaline and dopamine. Bath temperature 37° C. Every 3 min 0.5 μg histamine added. At N 0.2 μg noradrenaline, at D 20 μg dopamine added to bath. Between arrows 10^{-8} atropine present in the bath fluid. Pentolinium 5×10^{-6} present throughout. Bath vol. 10 ml.

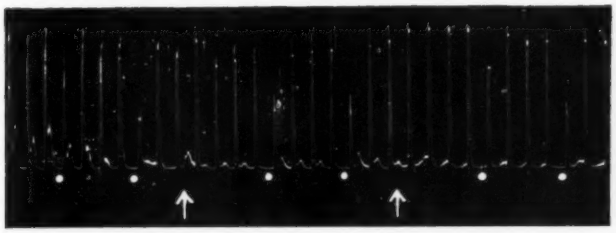

Fig. 8. Effect of cocaine on the inhibitory action of noradrenaline and dopamine. Bath temperature 37° C. Every 3 min 0.4 μg histamine added. At N 0.2 μg noradrenaline, at D 20 μg dopamine added to bath. Between arrows 10^{-5} cocaine present in bath fluid. Pentolinium 5×10^{-6} present throughout. Bath vol. 10 ml.

DISCUSSION

From the observation that bretylium, a drug shown by Boura & Green (1959) to inhibit specifically the release of noradrenaline, abolished the relaxation of the guinea-pig jejunum produced by mesenteric nerve stimulation, it can be concluded that the relaxation was the result of adrenergic nerve stimulation. The concentration and the time required to inhibit the adrenergic nerves of the guinea-pig jejunum were similar to those described by Boura & Green (1959) in the case of the rabbit ileum. The sensitization by cocaine to the response to adrenergic nervous stimulation has been observed in different organs (Trendelenburg, 1959; Hukovic, 1959). The enhancing effect of cocaine on the inhibition produced by

mesenteric nervous stimulation, therefore, also agrees with the conclusion that the relaxation was the result of stimulating adrenergic nerves.

Like bretylium, morphine and atropine were found to decrease the response to the adrenergic stimulation of the jejunum. As it has been shown in the second part of the experimental results, none of these drugs affected the depressant effect of noradrenaline sufficiently to explain this blocking action. The most likely interpretation of these results, therefore, is that the above drugs interfered in some manner with the release of noradrenaline from the nerve endings. The finding that the minimal concentration of morphine that inhibited the adrenergic nerves was about the same as required to reduce the effect of coaxial stimulation on the guinea-pig ileum, as reported by Paton (1957), would indicate that morphine interferes with a step common to cholinergic and adrenergic nerves. This minimal effective concentration is of the same order of magnitude as found in the brain of rats given a small analgesic dose of morphine (Adler, Elliott & George, 1957).

The depressant effect of morphine on the adrenergic nerves could be antagonized partially by nalorphine, whilst, according to Paton (1957), it was impossible to demonstrate conclusively the antagonism of nalorphine against morphine on the cholinergic nerves of the guinea-pig ileum. Although the optimal amount of nalorphine that antagonized the effect of any given concentration of morphine could not be established in this study, it could be clearly shown that nalorphine was effective against morphine present in a much higher concentration. This is in agreement with the *in vivo* findings of Orahovats, Winter & Lehman (1954), who reported that, in the rat, nalorphine antagonized the action of a 16 to 32 times greater dose of morphine. The antagonism of nalorphine to the depressant effect of morphine on the vagus of the rabbit has been also shown by Kosterlitz & Taylor (1959).

Atropine, in a small concentration, decreased the response to adrenergic stimulation without reducing the inhibitory effect of noradrenaline. The slow onset and disappearance of the nervous inhibition by atropine as compared to its antagonisms to acetylcholine suggest that the mechanisms of the two inhibitions are different.

From the observations that the depressant effect of dopamine was reduced when the temperature of the bath was lowered from 37° C to 28° C and that morphine inhibited the depressant action of dopamine only at 37° C, it can be concluded that dopamine acted in different ways at the two temperatures. At 28° C the nervous elements in the intestinal wall are not functioning and only the direct effect of dopamine on the adrenergic receptors could be observed which was not prevented by morphine. On the other hand, at 37° C dopamine acted, in a smaller concentration, on the adrenergic nerve endings, probably by releasing noradrenaline, and this effect was inhibited by morphine. Burn & Rand (1958b) have also shown that dopamine acts both by releasing noradrenaline and also directly on adrenergic receptors on the blood pressure of urethane-anaesthetized guinea-pigs, rabbits and cats.

The author wishes to thank the National Research Council of Canada for the support of the research presented in this paper. The careful assistance of Miss Betty Gannon and the generous supply of bretylium tosylate and hexamethonium chloride by Dr. J. R. Bogert, of Burroughs Wellcome & Co. (Canada), is gratefully acknowledged.

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SOME PHARMACOLOGICAL PROPERTIES OF *o*-METHYL-α-PROPYLAMINOPROPIONANILIDE, A NEW LOCAL ANAESTHETIC

BY

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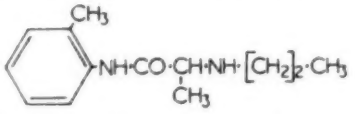
(*Received August* 29, 1960)

The anaesthetic action of *o*-methyl-α-propylaminopropionanilide (L 67, Astra) has been studied and compared with lignocaine in different *in vitro* and *in vivo* tests. Procaine and amethocaine (tetracaine) were also included in several of the comparisons. In nerve block on the isolated frog sciatic nerve L 67 is somewhat less effective than lignocaine. In all *in vivo* conditions, however, L 67 is quite comparable to lignocaine as to duration, latency and frequency of anaesthesia. As a spinal anaesthetic in rabbits 2 to 4% solutions of L 67 give a longer duration of anaesthesia than lignocaine. Toxicity in mice and rabbits and respiratory and circulatory effects in cats have also been evaluated. L 67 is almost twice as well tolerated as lignocaine. It is concluded that L 67 may prove to be a useful anaesthetic in certain clinical applications.

In recent years investigations of compounds chemically similar to lignocaine have been initiated because of the favourable clinical results obtained with this local anaesthetic. In collaboration with Löfgren and his associates a large number of lignocaine analogues have been synthesized and tested in our laboratories. One such compound is *o*-methyl-α-propylaminopropionanilide (L 67, Astra) (Löfgren & Tegnér, 1960), of which the chemical formula is:



Basic pharmacological data on this substance have been published by Wiedling (1960). In the study presented here the local anaesthetic properties of L 67 have been investigated *in vitro* (isolated frog nerve) and *in vivo* (nerve block, surface and spinal anaesthesia) in rats and rabbits. The action on the circulation and respiration and the toxicity of the drug have also been evaluated.

METHODS

Isolated nerve preparation. The sciatic nerve of the frog (Rana esculenta) and toad (Bufo vulgaris) was studied according to the method described by Mauro, Truant & McCawley (1948) and Truant (1957). The nerve was dissected out to include the tibial branch, and a 1.5 cm long portion of the nerve between the proximal stimulating and distal recording electrodes was immersed in a 4 ml. bath containing the local anaesthetic at 20° C. Supramaximal stimulation was performed with a Grass stimulator (Model S4E) including a stimulus isolation unit. The duration of the monophasic pulses was 0.05 msec and the voltage approximately 2.5 V.

A stimulation frequency of 30/sec was used and has been found to be preferable to single shock and higher frequencies in this type of studies. The compounds were dissolved as hydrochlorides in the modified Ringer solution used by Mauro *et al.* (1948) at *p*H 7.20.

The decrease in amplitude of the action potential (A-spike) was tested by applying the stimulus (30/sec) for 1 to 2 sec first every min while the test segment of the nerve was being bathed in the local anaesthetic solution and then every second min during washing out of the local anaesthetic. The action potential was recorded each time and 10 to 13 such recordings were obtained in the same picture by advancing the beginning of the sweep to the right between each exposure (see Fig. 1). The preparation was given 20 to 30 min rest after complete recovery. With this procedure the nerve could be used 6 to 8 times, alternately using test solutions of L 67 and lignocaine solutions as standards.

Nerve block in vivo. Female Sprague Dawley rats (150 to 200 g) were injected at the level of the mid-thigh using a 26 gauge 6 mm long needle and a volume of 0.2 ml. of the anaesthetic agent. For each compound half of the animals were injected in one leg with the test solution and, after recovery on this side, the other leg was injected with the standard (lignocaine) of corresponding concentration. In the remaining cases the reverse order of injection was used. Thus in this series each animal served as its own control. Six animals were injected in rapid succession and the motor paralysis was checked 3 min after the injection. If complete inability to make a foothold on a slanting net had developed, the symptom was checked every 5 min until complete recovery. Only complete paralysis was recorded as a positive result (block). The anaesthetics were used as hydrochlorides (*p*H 6.5 to 6.8). The method, described by Truant (1958), is similar to a previously developed technique for guinea-pigs (Shackell, 1935).

Test for topical anaesthetic action. The method of Nieschulz, Hoffman & Popendiker (1958), slightly modified, has been outlined elsewhere (Åström and Persson, 1961). One nostril of the rabbit was sprayed with 0.5 ml. of the anaesthetic agent while the other was used as control. The sneeze reflex was tested with a fine painter's brush. The reflex was considered blocked if it could be elicited neither at 1 nor at 3 min after the topical application of the local anaesthetic.

Spinal anaesthesia. Rabbits, weighing 2.0 to 2.5 kg, were used and 0.50 ml. of the anaesthetic solutions was injected through a 24 gauge needle between the 6th and 7th lumbar segments. The rabbits were restrained in a hammock. The method is essentially the same as that described by Bieter, Cunningham, Lenz & McNearney (1936). Anaesthesia was considered positive only if complete bilateral paralysis of the hind legs developed. The end-point of duration was determined by timing the first retraction response to pulling out of the legs while in the prone position.

Respiratory and circulatory effects. The action of L 67 on the systemic circulation was studied in cats anaesthetized with pentobarbitone sodium, 35 mg/kg injected intraperitoneally, by recording (1) respiration (pneumotachogram), (2) systemic blood pressure, (3) heart rate (e.c.g.) and (4) changes in peripheral resistance (hind leg) by observing pressure changes distal to a constant flow rate pump (Model T-8, Sigmamotor, Inc., N.Y.). Strain gauge pressure transducers were used and recordings made on a direct-writing Grass oscillograph. The anaesthetics were given intravenously as rapid injections (10 sec) or as infusions by a machine for 5 to 10 min.

Acute toxicity and tolerance. In albino mice the lethal dose was determined by continuous intravenous infusion (Hint and Richter, 1958; Richter, 1958). Using a constant infusion rate of 0.067 ml./min, the lethal dose was determined in 10 animals for each of 6 concentrations. Almost continuous diuresis was observed during the infusion of the large volumes. When the animal had lost its righting reflex death was determined at the time when all regular e.c.g. complexes (lead I) ceased. If death did not ensue the infusion was stopped after 2 hr (8 ml.).

Rabbits were given a series of single injections into the marginal ear vein in 30 sec and the dose selected such that the first injection produced a loss of the righting reflex (LRR) for a few min. The injections were repeated at 10 or 15 min intervals. Different doses were tested in series of 5 animals and 5 or 9 consecutive injections were made in each animal.

The local anaesthetic agents were used as hydrochlorides. A stock solution of 100 mM was prepared with 0.35% NaCl and dilutions made with 0.85% NaCl solution.

The ± values which follow the means in the presentation of data represent standard error of the mean. Significance of differences observed was tested by Student's *t* test (Snedecor, 1956).

RESULTS

Nerve block in vitro. A measure of the local anaesthetic activity was obtained by observing the reduction in height of the action potential produced in 5 min with different concentrations of L 67 and lignocaine. The choice of the exposure time of 5 min was considered practical for the later comparison with the *in vivo* tests. Substances which require a longer time to produce a block in this preparation would, *in vivo*, probably be removed from the injection site by the circulation, unless a vasoconstrictor was used, before a blocking concentration could be reached in the nerve structure.

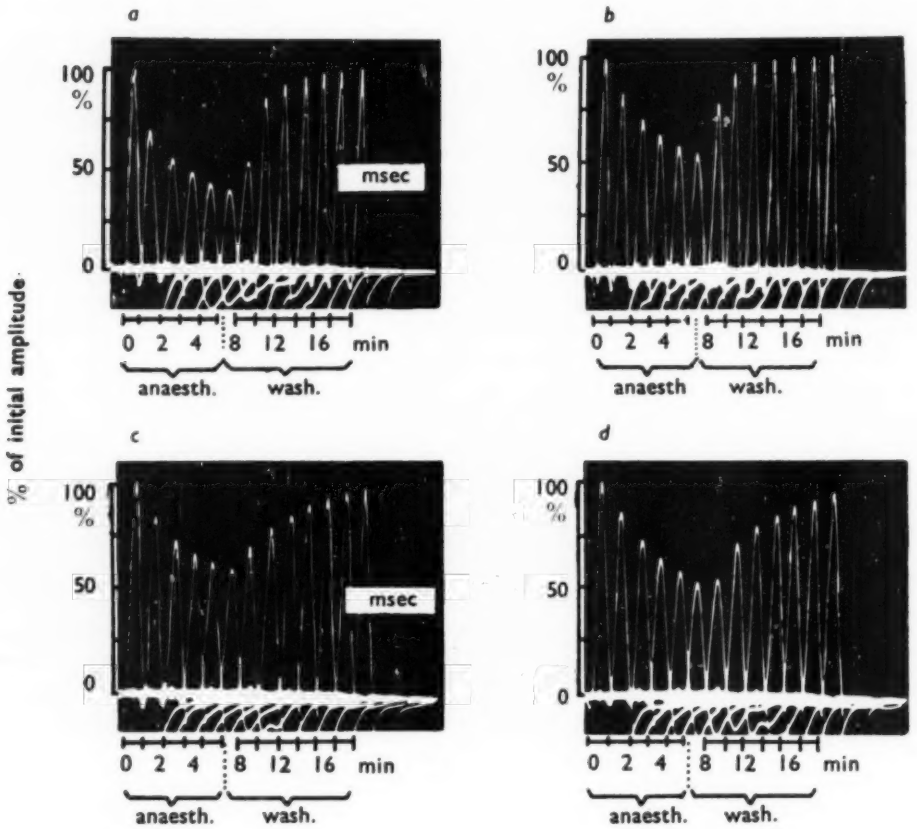


Fig. 1. Isolated frog sciatic nerve preparation. Decrease in amplitude of the action potential produced by (*a*) lignocaine, 5 mM, (*b*) L 67, 5 mM, (*c*) lignocaine, 5 mM, (*d*) L 67, 10 mM. The test segment (1.5 cm) of the nerve was immersed in the anaesthetic solution for 5 min and then washed with Ringer solution. The action potential was photographed each min while the agent was acting and every second min during washing. The beginning of the sweep was advanced to the right between each exposure. Stimulation frequency 30/sec. All solutions were *p*H 7.20.

On each nerve preparation the L 67 tests were always interposed between two lignocaine tests since the sensitivity of the nerve to the action of a local anaesthetic regularly increased during an experiment. The mean value of the bracketing lignocaine tests was used for comparison with the L 67 action. If tested in concentrations of 5 to 10 mM solutions L 67 was regularly less effective than lignocaine in reducing the height of the action potential (Fig. 1). On the basis of the results obtained in 12 nerve preparations it was concluded that in this preparation an 8 to 10 mM solution of L 67 is as effective as a 5 mM lignocaine solution.

L 67 and lignocaine were also studied with the same technique as in Fig. 1 on desheathed frog sciatic nerves. This preparation was about 5 times more sensitive than the intact nerve, but the relation between L 67 and lignocaine remained the same.

In similar experiments a 50% reduction of the amplitude of the action potential was produced by L 67 and lignocaine and the time for complete recovery was then measured. Using 5 mM lignocaine and 8 mM L 67 to produce the same degree of block, no difference in recovery time could be found. The recovery time of the action potential, during washing, from 50% to 100% of its original magnitude was 15 to 20 min for both agents. This is in sharp contrast to the recovery after amethocaine which under similar conditions is in excess of 1 hr as also found by Truant & Takman (1959).

Nerve block in vivo. 0.2 ml. of 0.5, 1.0 and 2.0% solutions of L 67 and lignocaine were injected in close proximity to the sciatic nerve in rats. The animals served as their own controls and the results (Table 1) show that the frequency and duration of

TABLE 1

NERVE BLOCK, SCIATIC NERVE IN RATS

Results from two series in which each animal received L 67 on one side and either lignocaine or procaine on the other

Compound	Concn. %	Duration of anaesthesia in min Mean±s.e.	Frequency of motor block out of 12
L 67	2	96±6	12
Lignocaine	2	92±5	12
L 67	1	80±5	12
Lignocaine	1	44±11	8
L 67	0·5	37±7	9
Lignocaine	0·5	28±7	8
L 67	2	105±5	12
Procaine	2	68±8	11
L 67	1	79±8	11
Procaine	1	6±2	6
L 67	0·5	52±8	10
Procaine	0·5	0±0	0

block were of the same order of magnitude for both local anaesthetics, although a slightly longer duration of anaesthesia was seen consistently with L 67 in these clinically relevant concentrations. The latent period for both substances is quite short (3 to 4 min). For purpose of comparison the results from a similar study with procaine have been included in Table 1.

Topical anaesthetic potency. The concentration needed of L 67 and lignocaine to block the sneeze reflex in 50% of the cases (EC50) was determined in rabbits. The EC50 for L 67 was $1.1 \pm 0.1\%$ (n=70) and for lignocaine $0.9 \pm 0.1\%$ (n=84). The EC50 and its standard error were estimated by the graphical method of Miller & Tainter (1944). No statistically significant difference in efficiency of L 67 and lignocaine was observed.

Spinal anaesthesia. The results at different concentrations are given in Table 2 and the relation between duration of anaesthesia and concentration of the anaesthetic

TABLE 2
SPINAL ANAESTHESIA IN RABBITS

Compound	Concn. %	*p*H	Duration of anaesthesia in min Mean±s.e.	Frequency of paralysis of hind legs
Lignocaine	4	6·6	44±5	10/10
	2	6·9	39±3	11/11
	1	6·8	31±6	11/11
	0·5	6·8	5±3	3/10
L 67	4	6·6	68±6	11/11
	2	6·6	46±4	10/10
	1	6·5	19±2	10/10
	0·5	7·0	5±2	6/10
Amethocaine	0·5	7·1	135±6	5/5
	0·25	7·0	76±16	5/5
	0·125	7·2	30±8	4/5

solutions presented in Fig. 2. At the lowest concentrations incomplete anaesthesias were noted with all agents and these cases were recorded as failures. At higher concentrations L 67 tended to produce anaesthesia of a longer duration than lignocaine. With 4% solutions this difference is statistically significant ($P<0.001$).

Both L 67 and lignocaine have a very short latent period and complete paralysis was generally seen as soon as the animals were removed from the hammock. The latent period of amethocaine was noticeably longer. In Fig. 2, for comparison, the dose-response curve for amethocaine is included, and the finding that it produces anaesthesia of a long duration at low concentrations is in agreement with previous investigations.

Action on circulation. In the cat under pentobarbitone anaesthesia, rapid intravenous injection of 1 to 3 mg/kg of L 67 produced a transitory fall in systemic blood pressure and usually some decrease in respiration of short duration. A fall in blood pressure has previously been observed in rabbits (Wiedling, 1960). The effects observed with L 67 on respiration and blood pressure were qualitatively the same as for lignocaine but less pronounced (Fig. 3A).

If high doses were given rapidly intravenously a secondary rise in blood pressure was sometimes observed. For lignocaine (6 to 8 mg/kg) this secondary rise was seen more clearly than with L 67 (Fig. 3B) and seems to result, at least in part, from changes in peripheral resistance. The respiratory changes in such cases are very pronounced, and the long-lasting apnoea in Fig. 3B suggested that the decrease in respiration might be the cause of the secondary increase in blood pressure and

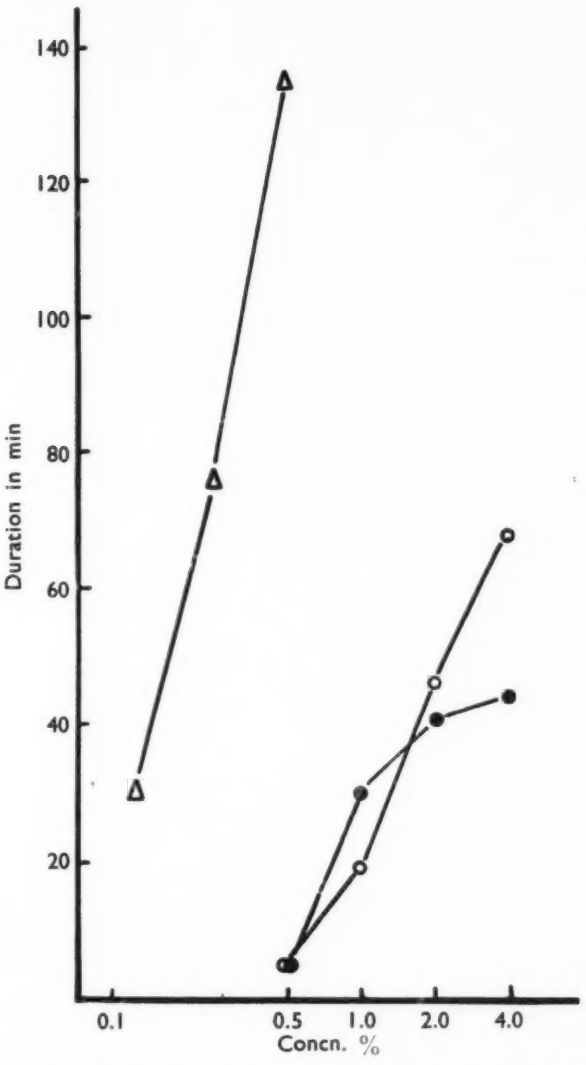


Fig. 2. Spinal anaesthesia in rabbits. Mean duration of anaesthesia at different concentrations. At high concentrations L 67 (○——○) produces anaesthesia of a significantly longer duration than lignocaine (●——●) ($P<0.001$ at 4%). △—— △=amethocaine. See also Table 2.

peripheral resistance. This assumption is supported by the results in Fig. 3C which shows that when hypoxia was prevented by artificial respiration the same high doses of lignocaine and L 67 produce predominantly a fall in blood pressure and peripheral resistance.

The effects of rapid intravenous injections of L 67 were compared with those of lignocaine in 5 cats, 2% solutions being given in different orders. From these studies it was concluded that in the dose range 2 to 5 mg/kg the depressor action of L 67 on (1) respiration is about half and (2) on blood pressure about half that of lignocaine. When hypoxia was prevented by artificial respiration the vasodilator effect of lignocaine, as measured in the hind leg, was about twice that of L 67.

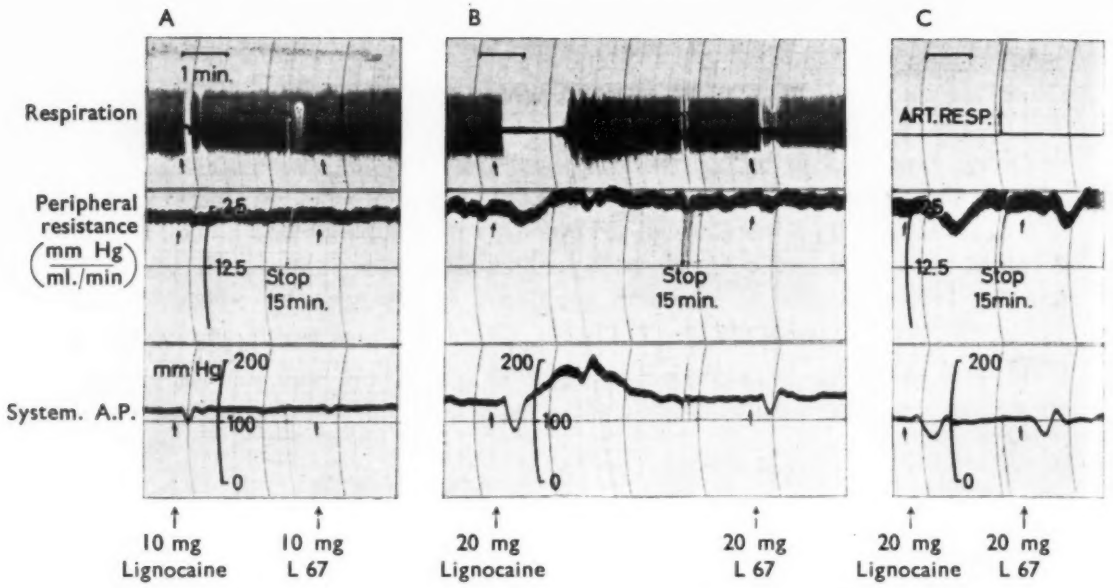


Fig. 3. Cat, 2.5 kg, pentobarbitone sodium anaesthesia. Effect of rapid intravenous injections of different doses of L 67 and lignocaine. Upper tracing, respiration; middle tracing, peripheral resistance in a hind limb measured by recording pressure changes distal to a constant flow rate pump; lower tracing, arterial blood pressure. Between A and B and B and C was a 30 min recovery period.

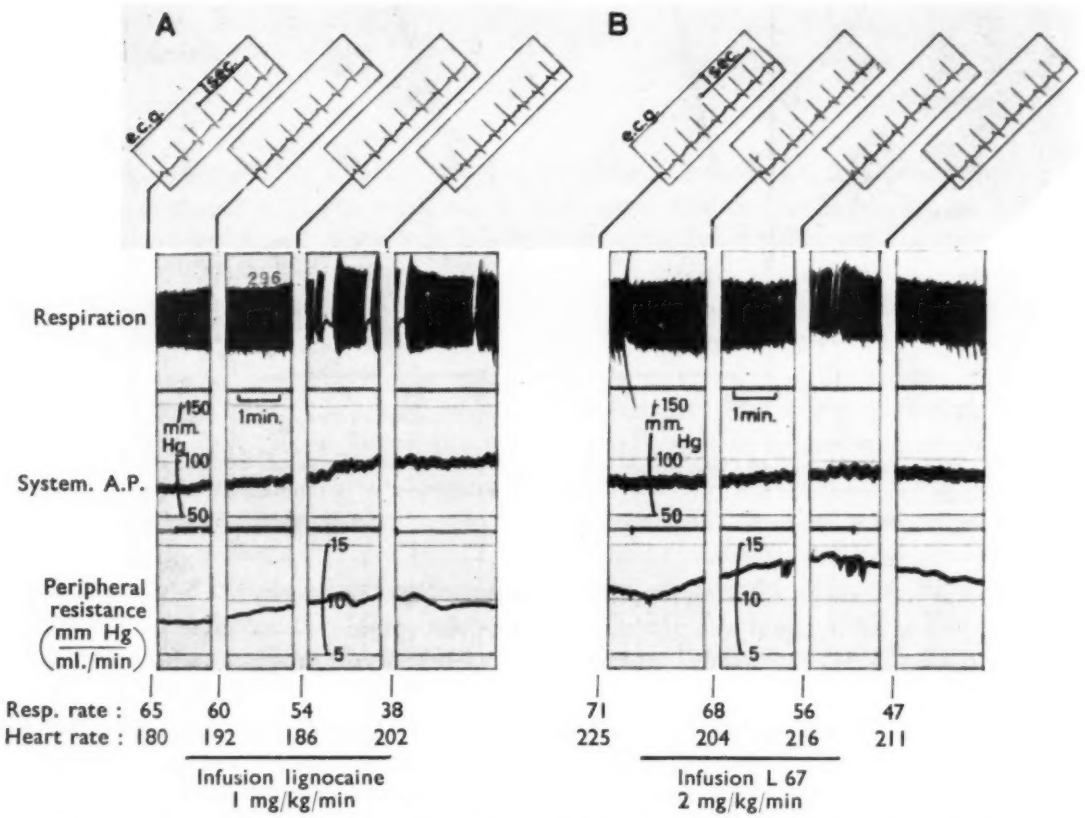


Fig. 4. Cat, 4.2 kg, pentobarbitone sodium anaesthesia. Effect on respiration, systemic blood pressure and peripheral resistance of intravenous infusions for 5 min of L 67 and lignocaine. The e.c.g. recording (top) and the respiratory frequency and heart rate/min (below) are given for each 5 sec period during which the speed of the polygraph was increased. Between A and B 45 min recovery.

In contrast to the results obtained with rapid intravenous injections an infusion of L 67 for several min usually produced a moderate increase in systemic blood pressure and peripheral resistance (Fig. 4B). At a rate of 2 to 3 mg/kg/min respiration is only moderately depressed. Lignocaine at only half this rate of administration produced a more marked depression of respiration and often produced repeated episodes of apnoea (Fig. 4A). As in the case of rapid injections of large doses the elevation in blood pressure seemed to follow largely as a consequence of respiratory depression and could be counteracted by artificial respiration. Other factors may, however, also contribute to the pressor effect on blood pressure. An elevation of blood pressure and cardiac output due primarily to a central effect has previously been demonstrated in dogs (Kao & Jalar, 1959). At infusion rates used in the type of experiments illustrated in Fig. 4 no definite changes were observed in the electroencephalogram for either L 67 or lignocaine.

Since in the cat experiments L 67 was found to produce less vasodilatation than lignocaine, it seemed possible that L 67 would enhance local circulation at an

TABLE 3

LETHAL DOSE DETERMINED BY CONTINUOUS INTRAVENOUS INFUSION

Solutions of different concentrations were infused at 0·067 ml./min until death occurred up to a maximum of 2 hr. With the lowest concentration of L 67 and amethocaine 1 of the 5 animals did not die within 2 hr

Compound	Concn. mM	No. of mice	Amount infused 10^{-6} mole/min/20 g body weight	Lethal dose mg (base)/kg Mean±s.e.
Lignocaine	100	10	6·70	190±22
	50	10	3·35	139±9
	25	10	1·67	167±17
	12·5	10	0·83	225±23
	6·25	5	0·42	266±14
L 67	100	10	6·70	190±34
	50	10	3·35	155±9
	25	10	1·67	222±19
	12·5	10	0·83	342±10
	6·25	5	0·42	>450
Amethocaine	6·25	10	0·42	28±1·3
	3·125	10	0·21	27±1·7
	1·563	10	0·11	50±3·3
	0·782	5	0·05	>75
Procaine	50	10	3·35	246±9
	25	10	1·67	180±6
	12·5	10	0·83	185±12
	6·25	10	0·42	236±35

injection site less than lignocaine. We are very grateful to Mr. Walde of this laboratory, who tested this possibility. He prepared 1% solutions of the agents with partially radioactive 0.85% NaCl solution and studied the clearance of ^{24}Na from the immediate surroundings of the sciatic nerve in rabbits. In two series of 6 animals it was found that ^{24}Na rose more slowly in the blood in the L 67 series than in the lignocaine series.

Toxicity and tolerance. The results from the infusion studies in mice are given in Table 3 and Fig. 5, in which the LD has been plotted against rate of infusion. The figure shows that with all anaesthetics the lethal dose decreases with increasing

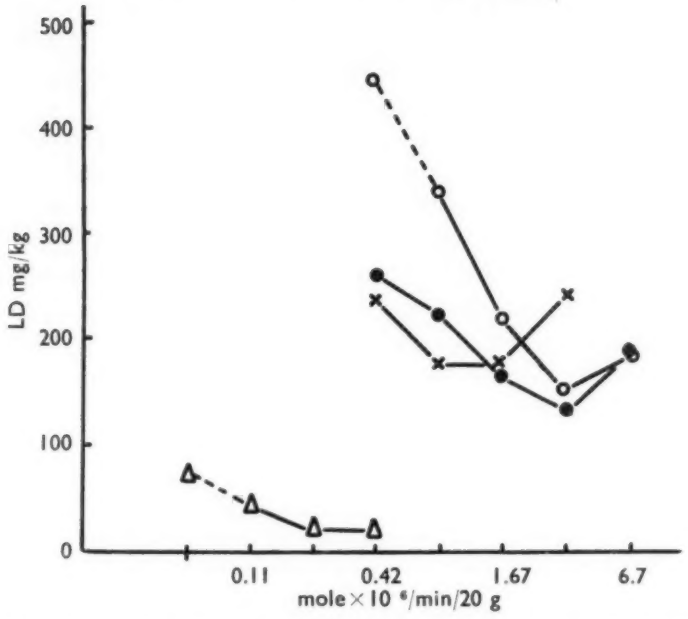


Fig. 5. Mice, 18 to 23 g. The lethal dose (LD), expressed in mg of the base, determined for L 67 (○——○), lignocaine (●—— ●), procaine (X——X) and amethocaine (△—— △) at different rates of intravenous administration. Infusions with different concentrations made at a constant volume rate of 0.067 ml./min. At 0.42×10^{-6} mole/min/20 g 1 of 5 animals receiving L 67 survived the 2 hr infusion of a total volume of 8 ml. With lignocaine and procaine survivals during the 2 hr started to occur at 0.21×10^{-6} mole/min/20 g and a valid estimate of LD was therefore not obtained at this rate of administration.

amount infused/unit time. Using 50 mM solutions (3.35×10^{-6} mole/min), the ratio LD lignocaine/LD L 67 was 0.9, while with 6.25 mM solution (0.42×10^{-6} mole/min) the ratio was about 0.6. At this latter concentration death occurred with L 67 in 4 of 5 cases. The animal which survived was infused with a total of 8 ml. in 2 hr, the others in this group died within 71 to 103 min. With lignocaine at the same rate of infusion all 5 animals died within 38 to 78 min, and with procaine all 5 died within 30 to 52 min. Since death was not observed within 2 hr L 67 could not be tested in the lowest concentration. With lignocaine and procaine survivals for 2 hr started to occur at the infusion rate of 0.21×10^{-6} mole/min. A valid estimate of LD was therefore not obtained at this rate of administration although a tendency for the procaine and lignocaine lines to cross was obvious. When 100 mM solutions were used the lethal doses were higher than those observed with 50 mM solutions of L 67 and lignocaine. Because these doses are very high and above the dose-response range, the results are not considered relevant here.

In the rabbit, tolerance for L 67 was compared with that for lignocaine. The study was performed by injecting intravenously at 10 and 15 min intervals a dose of each anaesthetic which on the first injection caused a loss of righting reflex (LRR). After preliminary trials two doses of 2% solutions were selected for the comparison, 9 and 12 mg/kg of lignocaine and 12 and 16 mg/kg of L 67. The injections were repeated if the animals had recovered from the preceding injection ; if no recovery occurred, then the test on this animal was terminated. Fig. 6 summarizes the results obtained.

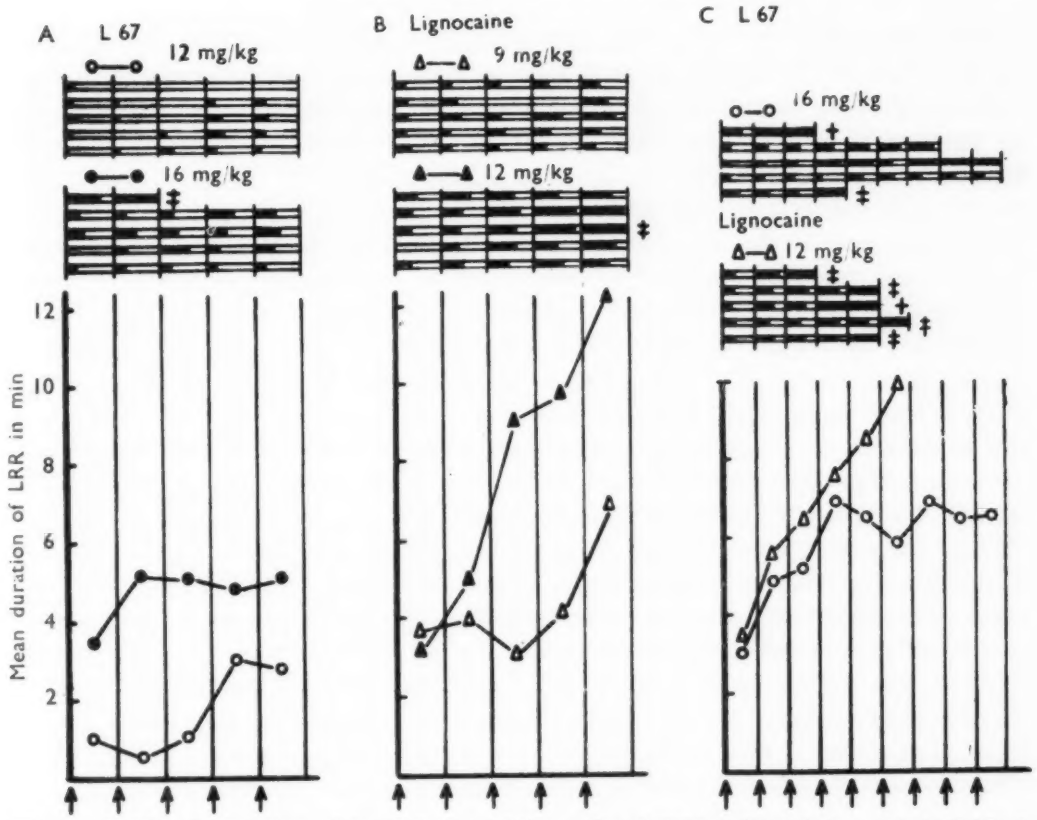


Fig. 6. Rabbits. Duration of loss of the righting reflex (LRR) indicated for each individual animal by black parts of the horizontal bars and the mean duration for each interval shown by the curves. Injections at 15 (A and B) and 10 (C) min intervals. † Death. ‡ Continued LRR.

The horizontal bars represent the duration of loss of the righting reflex (LRR) for each animal while the curves represent the mean duration for each group of 5 animals. In the cases when recovery had not taken place the duration of LRR for that interval was taken as either 10 or 15 min depending upon the intervals used. This tolerance study indicates that when lignocaine, 9 and 12 mg/kg, is injected at 15 min intervals (Fig. 6B) the duration of the LRR becomes progressively longer with each subsequent injection. Such an additive effect is not seen with L 67 (Fig. 6A) even at the high dose of 16 mg/kg. This difference between L 67 and lignocaine is well brought out in Fig. 6C in which 12 mg/kg of lignocaine and 16 mg/kg of L 67 are injected at 10 min intervals. Since the injected doses used are quite high and not much lower than the LD50 values for both anaesthetics some deaths were observed.

DISCUSSION

The methods used in this study were selected to determine certain pharmacological properties of L 67, which were thought to be related to important parameters in clinical anaesthesia. The investigation therefore includes (1) different methods of determining local anaesthetic activity, (2) various methods of evaluating systemic tolerance when administered intravenously at different rates, and (3) an evaluation of the effect on circulation and respiration.

Local anaesthetic action. The local anaesthetic action has been studied in a "semistatic" *in vitro* model, as well as in various dynamic *in vivo* conditions.

By the *in vitro* technique the efficiency of L 67 has been determined in relation to standard (lignocaine) by alternate tests on the same nerve preparation. The efficiency of a local anaesthetic determined by this method will largely be determined by its physico-chemical properties such as pK_a, lipid solubility and ability of the base to penetrate to the site of action in the nerve. The importance of these factors for the local anaesthetic effect has been discussed by Trevan & Boock (1927) and later by Ehrenberg (1948), Brown & Luduena (1953), Skou (1954) and Rud (1957). Determinations of certain physico-chemical properties of L 67 and lignocaine would therefore possibly offer an explanation for the lower activity of L 67 as observed on the isolated nerve preparation.

The dynamic *in vivo* conditions used were (1) nerve block, (2) topical anaesthesia of the nasal mucosa and (3) spinal anaesthesia. The experiments evaluating the ability of L 67 to block motor nerve fibres of the sciatic nerve in rats have shown that, at concentrations of 0.5 to 2.0%, L 67 is as effective as lignocaine in producing motor block. In these experiments it thus seems as if L 67 at the site of action reaches a sufficiently high concentration to make possible the same effective block in spite of its lower efficiency as determined on the isolated nerve.

The study of the topical anaesthetic potency of the two compounds on the nasal mucosa of the rabbit has also shown that L 67 and lignocaine are equally active. 2% solutions of L 67 and lignocaine produce topical anaesthesia of the same duration and in this respect both agents are comparable to 1% amethocaine on the nasal mucosa of the rabbit (Åström & Persson, 1961). Thus in this *in vivo* test, too, L 67 is as effective as lignocaine.

In both these physiological conditions (nerve block and topical anaesthesia) the effect of the agents upon local circulation is, in all probability, a significant factor. As shown in this study the vasodilatation produced in cats by lignocaine is greater than that of L 67. The difference in action on the local circulation has been illustrated by the finding that ^{24}Na is cleared more rapidly from a local site of injection when mixed with lignocaine than when mixed with L 67. If lignocaine is removed from the local site by the circulation more rapidly than L 67, this would explain why L 67 in these *in vivo* conditions is as effective as lignocaine in spite of its lower efficiency in the isolated nerve preparation.

The third dynamic *in vivo* situation studied was spinal anaesthesia. The dynamic situation here differs from the other *in vivo* conditions in that an injected compound can be expected to be slowly transported away from the site of application in the cerebrospinal canal. The results (Fig. 4) have shown that at higher concentrations L 67 gives an anaesthesia of a significantly longer duration than lignocaine. A consideration of the results by this *in vivo* test for the three anaesthetics leads to the following conclusions: Amethocaine penetrates slowly to the site of action (long latency observed here), but since transport from the spinal fluid is relatively slow the drug will stay locally long enough to make possible a sufficiently high concentration at the site of action. Once at the site, it is slowly removed and hence produces anaesthesia of long duration. Lignocaine penetrates rapidly (short latency), but, since it also can leave its site readily, its duration will

be limited by a relatively rapid transport away by the circulation in the nervous tissue. L 67, finally, also penetrates rapidly (short latency), and, even if it is not retained at the site of action any more firmly than lignocaine, its weaker enhancing action upon local circulation in the nervous tissue may retard its elimination by this route. The duration of anaesthesia by L 67 is therefore longer than for lignocaine in concentrations high enough to compensate for its lower efficiency as seen in the isolated nerve preparation.

Toxicity and tolerance. The methods selected to evaluate the toxicity of L 67 were designed to yield data which, if possible, should give some insight about the tolerance to L 67 to be expected under clinical conditions. For the clinical tolerance of a local anaesthetic in most applications and excluding intravenous usage, the usually determined intravenous toxicity would be of direct interest mostly in the rare instances of accidental intravascular injection. Normally the rate of absorption from an injection site is relatively slow and varies with the vascularity of the region and the effect of the anaesthetic upon local circulation. By the method used in this investigation with intravenous infusions at different rates, the conditions at the lowest rates of administration will simulate those when an agent is injected, for example, subcutaneously or intramuscularly, while at the highest rates the conditions are more like those of an ordinary intravenous toxicity study. The results (Fig. 5) have shown that if solutions of a high concentration are infused the lethal doses of L 67 and lignocaine in mice are quite similar. At lower rates of administration, however, mice tolerated L 67 much better than lignocaine. It may be added that due to the difference in action on peripheral vessels the rate of absorption of L 67 from an injection site can be expected to be slower than that of lignocaine in most clinical applications, if both agents are used without a vasoconstrictor and injected in the same concentration and volume.

A similar conclusion as to tolerance for L 67 was obtained by multiple intravenous single injections at 10 and 15 min intervals in rabbits. As shown in Fig. 6, L 67 was better tolerated than lignocaine. A more rapid destruction of L 67 than lignocaine would seem to be the most plausible explanation for this difference in tolerance. The experiments with rapid intravenous injections as well as infusions in cats have further illustrated that L 67 is well tolerated (Figs. 3 and 4) and the action of L 67 upon respiration and systemic blood pressure is regularly less pronounced than that of lignocaine.

With the appropriate caution with which experimental results on animals always should be regarded when used to predict clinical usefulness of a compound, we would like to conclude that due to its favourable local anaesthetic activity and tolerance L 67 may prove to be a useful anaesthetic in certain clinical applications. On the mucous membranes in the respiratory tract and probably elsewhere its effectiveness may be expected to be of the same order of magnitude as that of lignocaine. Like lignocaine it is less rapidly absorbed than amethocaine from mucous membranes in the rabbit (Åström & Persson, 1961). In the case of the anaesthetics without the addition of a vasoconstrictor it may be expected that L 67 for infiltration anaesthesia and nerve block (in sites comparable to the mid-thigh sciatic nerve preparation in the rat) will be as effective as lignocaine. This has been found to be true in finger nerve blocks (Eriksson & Gordh, 1959).

It remains to be determined, however, if the lower relative efficiency of L 67 in comparison with lignocaine, as found on the isolated nerve preparation, will make it inferior to lignocaine in sites where a more extensive and profound action is required. For certain clinical applications it should also be considered that the action of L 67 cannot be prolonged by the addition of adrenaline to the same degree as lignocaine. This difference was first observed by Berling & Björn (to be published) in their studies of dental infiltration (terminal) anaesthesia in man and has been confirmed in our laboratory on sciatic nerve block in rats.

We are grateful to Dr. A. P. Truant, Worcester, Mass., U.S.A., for many helpful suggestions.

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THE EFFECTS OF ION CHANGES ON THE CONTRACTION OF THE RAT UTERUS STIMULATED BY OXYTOCIN

BY

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(*Received August* 30, 1960)

The contraction of the rat uterus to oxytocin was measured when it was bathed in various solutions. Substitution of nitrate or bromide for two-thirds of the chloride present increased the size of contractions, while a similar substitution of sulphate and sucrose for chloride depressed them. Alteration of the ratio of chloride to sodium by substitution of choline for two-thirds of the sodium present enhanced the contractions, but this was probably due to the choline *per se* as choline sulphate similarly enhanced the response. Partial replacement of both sodium and chloride by sucrose did not alter the size of contractions.

The substitution of bromide, nitrate or iodide for the chloride in the bathing solution enhances the twitch tension of skeletal muscle (Hill & Macpherson, 1954) but reduces the size of isotonic contractions of cardiac muscle (Nayler & McCulloch, 1960). The effects of such anion changes on smooth muscle have not been reported, to our knowledge, but electrophysiological results suggested that such studies would be worth while. Burnstock & Straub (1958) have shown that smooth muscle is depolarized to a greater extent by potassium sulphate than by potassium chloride, and have suggested that chloride may be contributing to the membrane potential. It may be relevant in this regard that Daniel & Bass (1956) have found that there is a higher concentration of chloride in smooth muscle than in skeletal muscle. Although the role of cations in uterine smooth muscle contractility has previously been studied (see, for instance, Van Dyke & Hastings, 1928; Bentley & Dicker, 1955; and Munsick, 1960), it seemed that the effect of changes in the relative proportions of chloride and sodium would merit investigation, as Holman (1957) found with the taenia coli of the guinea-pig that tension and rate of spike discharge were increased when two-thirds of the sodium chloride in the bathing solution was replaced with choline chloride. Atropine did not abolish the effect, which was therefore presumably not due to an acetylcholine-like action of the choline. Reduction of both sodium and chloride by the use of sucrose instead of choline chloride had no such effect.

The present work reports the effects, on contractions of the rat uterus produced by oxytocin, of reducing chloride concentration in the bathing solution while leaving the sodium level unchanged, of substituting choline for sodium leaving the chloride unchanged and of replacing both sodium and chloride with either sucrose or choline sulphate.

METHODS

The uterus of the rat in oestrus was isolated and suspended in a 50 ml. bath containing a modified Locke solution (García de Jalón, Bayo Bayo & García de Jalón, 1945). The composition of this solution was: 9.0 g NaCl, 0.42 g KCl, 0.06 g $CaCl_2$, 0.5 g $NaHCO_3$, 0.5 g glucose and 1 l. water. The bath was kept at 29.5° C and gassed with oxygen. Contractions of the uterus to 3 different submaximal doses of oxytocin (Parke, Davis & Co.) were recorded while it was bathed with the modified Locke solution. The experimental solution was next run into the bath. At this stage spontaneous activity was often great. If, however, it was not sufficient to interfere with the responses to oxytocin 3 more doses identical with the previous ones were given in the same order. After 35 min this was repeated.

With the uteri that were initially contracting spontaneously, the lapse of 35 min was usually long enough for the contractions to subside, and the original 3 doses of oxytocin were again given. After the period in the experimental solution the uterus was again made to contract in the normal modified Locke solution to see whether the observed effects were reversible or not.

The results are expressed as the percentage change in response of the uterus in the experimental solution, compared to the response to the same dose of oxytocin given initially while the uterus was bathed with the standard modified Locke solution. The percentage changes with the 3 doses were pooled and averaged. The values were compared with the controls in which the modified Locke solution was used throughout the whole observational period, responses to oxytocin being measured at times corresponding to the successive tests in the experimental series.

The preliminary experiments on the permeability of the rat uterus in the various solutions are based on those of Conway (1957). Pieces of the uterus in oestrus were suspended in the experimental solutions at 2° C and weighed at intervals. The rates of swelling in the different solutions were compared to see whether there was any correlation between this rate and the contractile response.

RESULTS

Ion replacement. These results are in Table 1.

TABLE 1

THE EFFECTS OF IONIC CHANGES ON THE RESPONSE OF THE RAT UTERUS TO OXYTOCIN

The mean values (±s.e.) are given as percentage increase or decrease in response to oxytocin when de Jalon solution was replaced by experimental solution. In control experiments de Jalon solution was merely renewed.

Replacement of ⅔ NaCl in de Jalon solution by:	No. of expts.	% Increase (+) or decrease (−) in response to oxytocin: (i) Initially, in experimental solution	*P*	(ii) After 35 min in experimental solution	*P*
Sodium nitrate	10	+97·1±12·5	<0·001	+72·5±11·06	<0·01
Sodium bromide	10	+68·9±13·8	<0·001	+96·6±14·4	<0·001
Sodium sulphate +sucrose	10	−34·5± 4·3	<0·001	−57·4± 4·5	<0·001
Choline chloride	9			+100·9±15·4	<0·001
Sucrose	9			+8·1± 7·4	<0·05
Choline sulphate +sucrose	10			+142·1±17·7	<0·001
Choline bromide	10			+142·1±18·6	<0·001
Control	12	+0·7± 5·4		+27·0± 9·5	

Replacement of chloride. The sodium concentration was kept constant while the chloride was partly substituted with either nitrate or bromide. In both cases there was an immediate and reversible increase in contractions in response to oxytocin. If, however, sulphate plus sucrose was used as a substitute for chloride

there was a depression in the response which returned to normal when the sulphate plus sucrose was washed out of the bath.

Reduction of sodium concentration. The sodium concentration in the bathing solution was reduced to 0.3% while the chloride was left unchanged. Choline was substituted for the sodium. Contractions of the uterus increased markedly in response to oxytocin in this solution. Spontaneous contractions were observed initially. The increased response reverted to normal after washing out the choline. The response was unaffected by atropine sulphate (0.1 mg/l.).

In three experiments substitution of choline for all of the sodium except that present as bicarbonate (6 m-equiv/l.) resulted in spontaneous activity of the uterus and an increase in the size of the contractions produced by oxytocin, similar to that seen with $\frac{2}{3}$ substitution of choline for sodium. On returning the uterus to normal de Jalon solution, the activity returned to normal.

Reduction of sodium chloride concentration. Both the sodium and chloride of the bathing solution were reduced to $\frac{1}{3}$ the normal concentration. The reduction was effected by substitution of different solutes for the sodium chloride. If sucrose was used there was an increase in the spontaneous activity, but no change in the response to oxytocin. On the other hand, the substitution of choline sulphate plus sucrose for the sodium chloride not only increased spontaneous activity but also increased the response to oxytocin. Sucrose was used to adjust the osmotic concentration as sulphate is divalent. If choline bromide was used as a substitute for sodium chloride, there was also a marked increase in the response to oxytocin, which reverted to normal when the uterus was replaced in de Jalon solution.

Tissue swelling. The rates of swelling of the uterus when bathed in various solutions kept at 2° C are shown in Table 2. It can be seen that while there were

TABLE 2

THE RATE OF SWELLING OF THE UTERUS IN DIFFERENT SOLUTIONS

Results are given as the difference between the % swelling in the experimental solution and the % swelling in normal de Jalon solution. The figures below represent the mean ±s.e. in 6 experiments. A negative value indicates less swelling than the control. A positive one indicates more swelling. Sucrose was the only substitution that resulted in an absolute shrinkage of the uterus

Replacement of $\frac{2}{3}$ NaCl in de Jalon solution by:	1 hr	6 hr
Sodium sulphate +sucrose	−0·40±0·92	−0·74±0·92
Sodium bromide	−0·46±0·74	+1·71±0·89
Sodium nitrate	−2·18±1·28	−3·39±1·82
Sucrose	−7·37±1·10	−20·12±1·80
Choline chloride	−0·97±0·50	−6·17±1·69
Choline sulphate +sucrose	−0·02±0·94	−5·57±1·42

some marked differences in the rates of swelling in the different solutions, these were not correlated with differences in the contractile responses to oxytocin. For example, there were no significant differences between shrinkage in sodium sulphate plus sucrose and that in sodium bromide or in sodium nitrate, yet the last two enhanced the response to oxytocin while the sulphate depressed it.

It is interesting that the swelling of the pieces of uterus kept in solutions of low sodium concentration were far less than those in solutions of normal sodium concentration, which is consistent with the results of Leaf (1956) on the swelling of rat liver slices.

DISCUSSION

The present results indicate that anions play an important part in the response of uterine smooth muscle to oxytocin when the sodium concentration is unchanged. The depressant effect of sulphate might have been due to a relative impermeability of the muscle cell membrane to this ion or to a reduction in ionized calcium. The latter cannot definitely be excluded, but it may be noted that a similar increase in sulphate was not depressant in experiments with choline salts. The enhancement of contraction when $\frac{2}{3}$ of the chloride was replaced with bromide or nitrate is consistent with the results in skeletal muscle (Hill & Macpherson, 1954) but contrasts with those in cardiac muscle (Nayler & McCulloch, 1960). In skeletal muscle the increase in twitch tension seen in the presence of bromide or nitrate has been correlated with a prolonged after-potential (see Harris, 1958) and this prolonged after-potential is also seen in the presence of choline (Edwards, Ritchie & Wilkie, 1956). As the tension produced in smooth muscle is known to be related to the rate of spike discharge (Bülbring, 1958), it would be interesting to know the effects on smooth muscle of nitrate or bromide on the rate of spike discharge as well as the action potential, and see if this could be correlated with the changes in contractility.

The results with sucrose and with choline salts do not allow a clear distinction to be drawn between the possible effects of choline *per se* and of disturbances in the ratio chloride to sodium.

The enhancement of contractions with choline salts whether as chloride *or* sulphate, however, favours an effect by choline. It will be noted that this was not abolished by atropine. Possibly the membrane is more permeable to choline than sodium, but this is not indicated by the rate of swelling of the uterus in choline as compared to sodium solutions.

This work was carried out with the aid of a Medical Research Grant from the University of Western Australia. Mr. N. Stenhouse of the C.S.I.R.O. kindly performed the statistical analysis of the results. We are grateful to Professor W. J. Simmonds for useful discussion of the results.

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EFFECT OF SEX HORMONES ON THE EXCRETION OF FREE HISTAMINE BY MALE AND FEMALE RATS

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(*Received September* 22, 1960)

The excretion of free histamine by male rats was increased 10-fold by treatment with oestrogen or castration. That of female rats was reduced to 1/10 by treatment with testosterone. Ovariectomy further increased the histamine excretion by female rats, which was slightly reduced by treatment with oestrogen, and at oestrus. The low level of free histamine excretion by male rats was not increased by compound SKF 525-A, and inhibition of diamine oxidase by aminoguanidine caused the same proportionate increase in both sexes. It is concluded that the conjugation of histamine by male rats requires the presence of androgens, and some preliminary indication is given regarding the possible mechanisms involved.

Leitch, Debley & Haley (1956) and Gustafsson, Kahlson & Rosengren (1957) reported that male rats excrete about 1/10 as much free histamine in the urine as females. Westling (1958) confirmed these observations and, studying the origin of this difference using [^{14}C]histamine, concluded that the male rats had a larger capacity to methylate histamine. The object of the experiments described in the present paper was to determine in what way, if any, this phenomenon was associated with the sex hormones.

METHODS

Urine collection. Rats of the Wistar strain, of both sexes, were used. They were placed in metabolism cages for the collection of serial 24-hr urine specimens. The cages, of galvanized wire mesh, were circular and of 25 cm diameter. Each cage was supported over a large polythene funnel, at the neck of which the conventional pear-shaped glass globe was suspended to separate urine from faeces. In the earlier experiments, urine was collected into bottles containing 2 g solid trichloracetic acid, but subsequently 0.1 to 0.5 ml. concentrated hydrochloric acid was used, according to the number of rats in the cage. The acidified urine samples were stored in the refrigerator at 4° C.

Extraction and assay of histamine. The urine specimens preserved with trichloracetic acid were shaken with three portions each of 4 vol. ether to remove the trichloracetic acid. In later experiments, this time-consuming procedure was avoided by using hydrochloric acid as preservative, when all that was necessary was to neutralize with a few drops of 20% sodium hydroxide. Where the final dilution for assay was several 100-fold, the acidity was adequately diluted and buffered out by the Tyrode solution, without previous neutralization. The amount of free histamine in the urine was determined by 4-point assay on isolated guinea-pig ileum suspended in Tyrode solution containing atropine sulphate, 10^{-7} g/ml., in an automatic apparatus.

In a few specimens, hydrolysable histamine was determined by refluxing a 5 ml. aliquot of the urine with 1.25 ml. concentrated hydrochloric acid under an air condenser for 90 min.

thereafter proceeding according to Code's modification (1937) of the method of Barsoum & Gaddum (1935) for blood histamine.

Drugs. Stilboestrol, B.P. (B.D.H.) (solution of 1 mg/ml. in oil); injection of oestradiol monobenzoate, B.P. (B.D.H.) (2 mg/ml. in oil); injection of testosterone propionate, B.P. (B.D.H.) (5 mg/ml. in oil); SKF 525-A, research sample of β-diethylaminoethyl diphenylpropylacetate, supplied by Smith, Kline & French Laboratories; aminoguanidine bicarbonate (May & Baker).

RESULTS

Effect of oestrogen and castration on histamine excretion of male rats

Two groups each of 6 male rats, of 100 g wt., were housed in two metabolism cages, and serial 24-hr urine specimens were collected. After a 6-day control period, group A received stilboestrol, 50 μg/rat/day subcutaneously for 12 days; group B, arachis oil, 0.05 ml./rat/day subcutaneously. After 6 days' latent period, the histamine excretion of the oestrogen treated group rose steeply from under 5 μg/rat/day to a peak of 44 μg, which is in the range of excretion for female rats. When the stilboestrol was stopped on the 18th day, the histamine excretion fell again, reaching the original level about the 38th day. The histamine excretion of the control group receiving arachis oil remained at the low male level (Fig. 1).

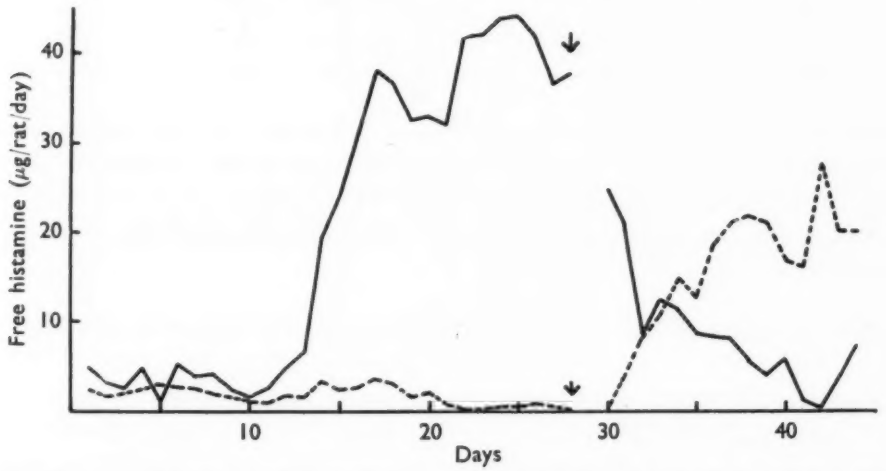

Fig. 1. Effect of stilboestrol and castration on the histamine excretion of male rats. Group A (continuous line): 6 rats treated with stilboestrol, 50 μg/rat/day subcutaneously, 7th to 19th day; "sham" operated 28th day (↓). Group B (broken line): 6 rats treated with arachis oil, 0.05 ml./rat/day subcutaneously, 7th to 19th day; castrated 28th day (↓).

On the 28th day, the rats in group B were castrated, under ether anaesthesia, by the method described by Burn (1950a). Those in group A were "sham" operated, that is, they received the anaesthetic and surgical treatment associated with the castration procedure, but without the testes being removed. Both groups were allowed 24 hr recovery period in ordinary cages on cotton wool before returning to the metabolism cages, and hence missed one 24-hr collection of urine. Immediately after castration, the histamine excretion of group B rose sharply, reaching a peak of 28 μg/rat/day 12 days after operation, and thereafter maintaining the high female histamine output indefinitely (Fig. 1).

Hydrolysable histamine in urine from male rats

In the previous experiment, aliquots from a few urine samples were subjected to hydrolysis, as described under "methods," before assay of histamine. The results are shown in Table 1. It will be seen that, in group A when the free histamine excretion was low, the amount of biologically detectable histamine was

TABLE 1

EFFECT OF HYDROLYSIS ON THE HISTAMINE CONTENT OF URINE FROM MALE RATS (FIG. 1)

Values for histamine expressed as μg/rat/day

	Day	Group A Free	Group A Hydrolysed (total)	Group A % hydrolysed free	Group B Free	Group B Hydrolysed (total)	Group B % hydrolysed free
	7	3·9	12·2	314			
	8	4·2	16·7	397			
Stilboestrol	15	24·0	25·5	106	2·2	10·5	475
	17	38·2	29·5	77	3·4	11·1	328
	19	32·4	26·0	80	1·6	5·0	310
	25	44·0	46·3	105	0·7	6·5	980
Castrated	39	4·0	18·0	450	21·0	28·3	135
	45	4·0	7·2	180	25·2	25·2	100
	56	3·0	9·1	303	32·3	29·2	90
	64	4·0	16·7	417	23·5	26·5	113

increased by 3 to 5-fold by acid hydrolysis of the urine, whereas during treatment with stilboestrol, when the free histamine level was raised, hydrolysis produced little or no increase in assayable histamine. The same decrease in proportion of hydrolysable histamine was seen in group B when the free histamine excretion rose after castration.

Effect of androgen, oestrogen and ovariectomy on the histamine excretion of female rats

The experiment was carried out in the same time sequence as the previous one, using female rats of 100 g. Group A received testosterone, 0.5 mg/rat/day subcutaneously, the control group B arachis oil, 0.1 ml./rat/day. One day after beginning this treatment, the histamine excretion of the testosterone treated rats fell sharply, reaching the low male level of less than 5 μg/rat/day, subsequently returning to the original level when treatment was withdrawn (Fig. 2).

On the 28th day, the rats in group B were ovariectomized, under ether anaesthesia, by the method described by Burn (1950b), and those in group A were "sham" operated. The histamine excretion of the ovariectomized rats did not fall, as expected, but rose higher, reaching a peak of 76 μg/rat/day (Fig. 2).

Fig. 3 is a continuation of the same experiment, and shows that the histamine excretion of both ovariectomized and "sham" operated rats was reduced by oestrogen, in this experiment, oestradiol benzoate. The ovariectomized rats were, however, more sensitive to oestrogen than the normals, since 50 μg/rat/day produced a larger fall of histamine excretion in the former than 100 μg/rat/day in the latter.

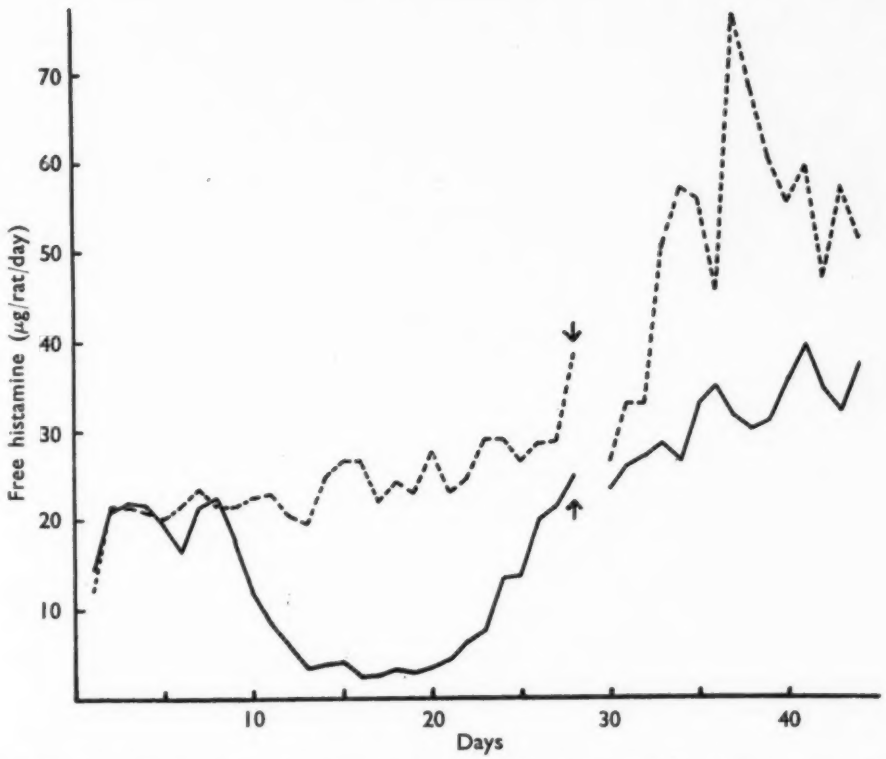

Fig. 2. Effect of testosterone and ovariectomy on the histamine excretion of female rats. Group A (continuous line): 6 rats treated with testosterone, 0.5 mg/rat/day subcutaneously, 7th to 19th day; " sham " operated 28th day (↑). Group B (broken line): 6 rats treated with arachis oil, 0.1 ml./rat/day subcutaneously, 7th to 19th day; ovariectomized 28th day (↓).

From these results, it appears that the ability of male rats to conjugate histamine is dependent upon the presence of androgen.

Histamine excretion and oestrus cycle in female rats

In view of the effects of ovariectomy and of oestrogens on histamine excretion already observed (Figs. 2 and 3), it seemed probable that there might be a fluctuation of histamine excretion in female rats in parallel with the oestrus cycle. To investigate this possibility, it was necessary to collect 24-hr urine specimens from one female rat, and Fig. 4 shows the results of such an experiment. The arrow shows the incidence of oestrus as indicated by cornified epithelial cells in the vaginal smear. Daily urine volumes are also recorded. It will be seen that there are well-defined troughs in the histamine excretion graph coincident with oestrus, which is in agreement with the previously observed depressant action of injected oestrogen. It was also noticed that the volume of urine excreted was depressed at oestrus. This may be related to the mild action of oestrogens in promoting sodium and water retention, first reported by Thorn & Engel (1938). Daily injections of 50 μg stilboestrol for 5 days produced a continuous condition of oestrus and a continuous depression of both urine and histamine excretion. When oestrogen was stopped,

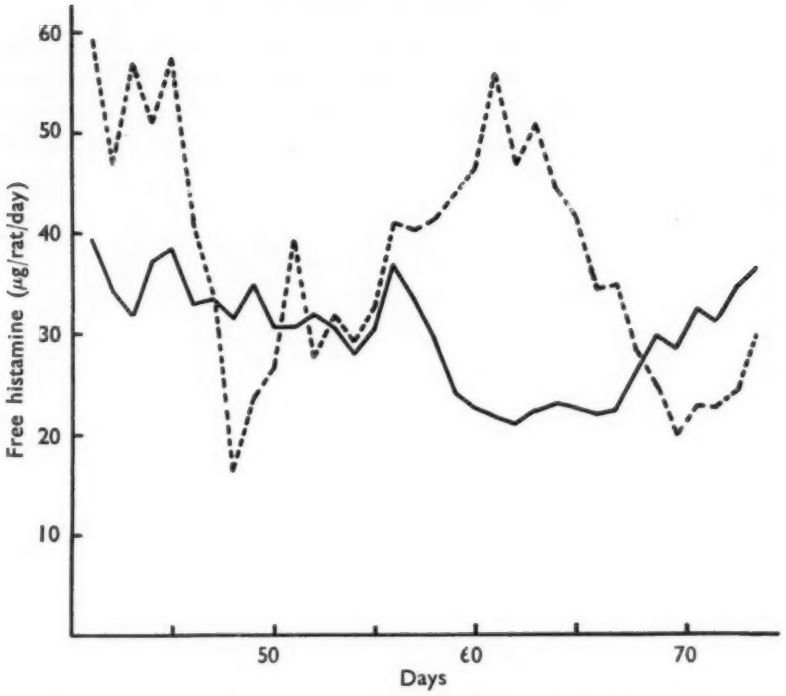

Fig. 3. Continuation of Fig. 2. Effect of oestradiol on the histamine excretion of female rats. Group A (continuous line): 6 "sham" operated rats treated with oestradiol, 100 μg/rat/day subcutaneously, 56th to 66th day. Group B (broken line): 6 ovariectomized rats treated with oestradiol, 50 μg/rat/day subcutaneously, 45th to 54th day.

histamine and urine excretion returned immediately to their original levels and cyclic fluctuations, though the oestrus cycle, as indicated by vaginal smears, was suppressed for 16 days, presumably by the depressant action of the exogenous oestrogen on the pituitary gland.

Development of sex difference in histamine excretion

To determine the age at which the histamine excretion by male and female rats becomes differentiated, 5 male rats and 5 females, litter-mates to the males, were placed in separate metabolism cages immediately after weaning at 20 days old. The histamine excretion of the two groups is shown in Fig. 5. The excretion curves begin to diverge after the 26th day of life. The males reached the characteristic low level by the 35th day, at about the time the testes descended. The females reached the high level of excretion at the 40th day of life, when the vaginae were opening. The first oestrus was recorded by vaginal smear on the 45th day, and 4 of the 5 rats had shown their first oestrus by the 48th day.

Site and mechanism of histamine conjugation in male rats

Conjugation mechanisms are enzymic processes and it should therefore be possible to prevent the conjugation of histamine by male rats by means of an

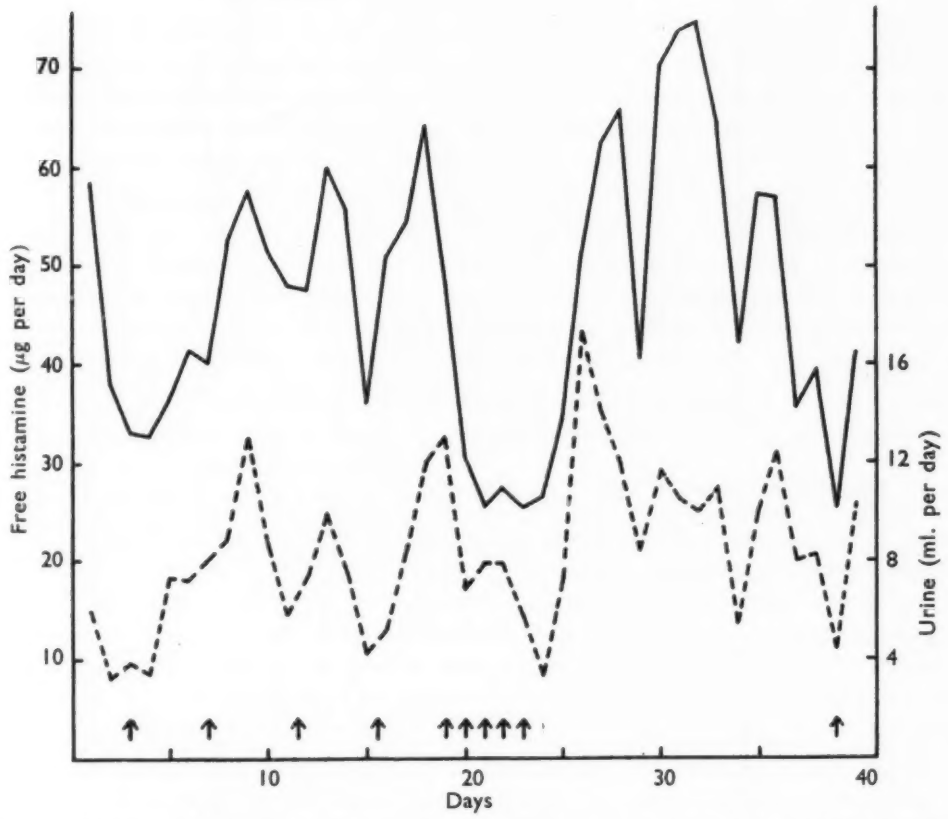

Fig. 4. Relationship between oestrus cycle and excretion of urine and histamine in one rat. Continuous line: histamine excretion; broken line: urine excretion. Arrows indicate incidence of oestrus. Stilboestrol, 50 μg/day from 18th to 23rd day.

appropriate enzyme inhibitor. Compound SKF 525-A is an inhibitor of a variety of detoxicating and conjugating mechanisms in the liver (Cooper, Axelrod & Brodie, 1954). Fig. 6 shows that, when this compound was injected into groups of male and female rats, it had no effect on the histamine excretion of either sex. In the same experiment, aminoguanidine, an inhibitor of diamine oxidase, produced about a 4-fold increase in the free histamine excretion of both males and females. This increase was due only to the blockade of histamine metabolism and not to specific blockade of conjugation by male rats, since the increase was proportionally the same in both sexes.

DISCUSSION

The failure of ovariectomy to produce a lowering of free histamine excretion in female rats indicates that the ability of male rats to conjugate most of their excreted histamine is not due to the absence of oestrogen. On the other hand, when female rats are given testosterone, their free histamine excretion falls to the male level, and when androgen production in males is suppressed, either by oestrogen administration or by castration, free histamine excretion rises to the female level. These observations indicate that the presence of androgen is an essential factor for the

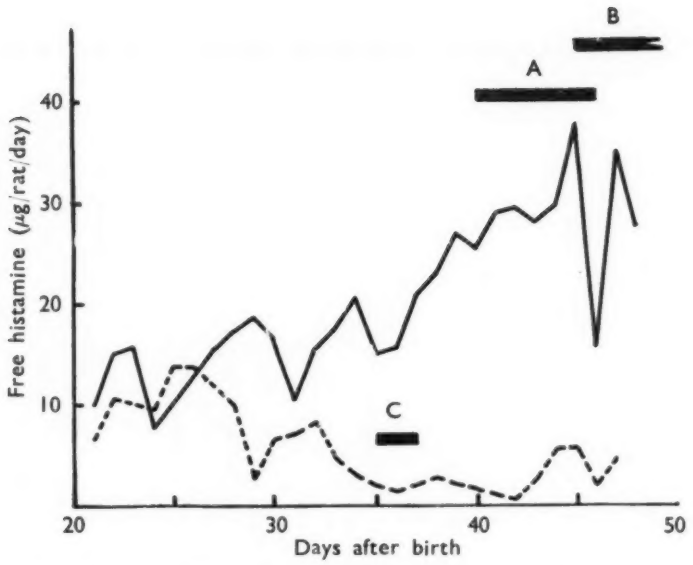

Fig. 5. Development of sex difference in histamine excretion in rats. Histamine excreted by 5 female rats (continuous line) and by 5 male rats (broken line). Bars A and B indicate the time of the opening of the vagina and the first oestrus respectively in the females and C the time of descent of the testes in the males.

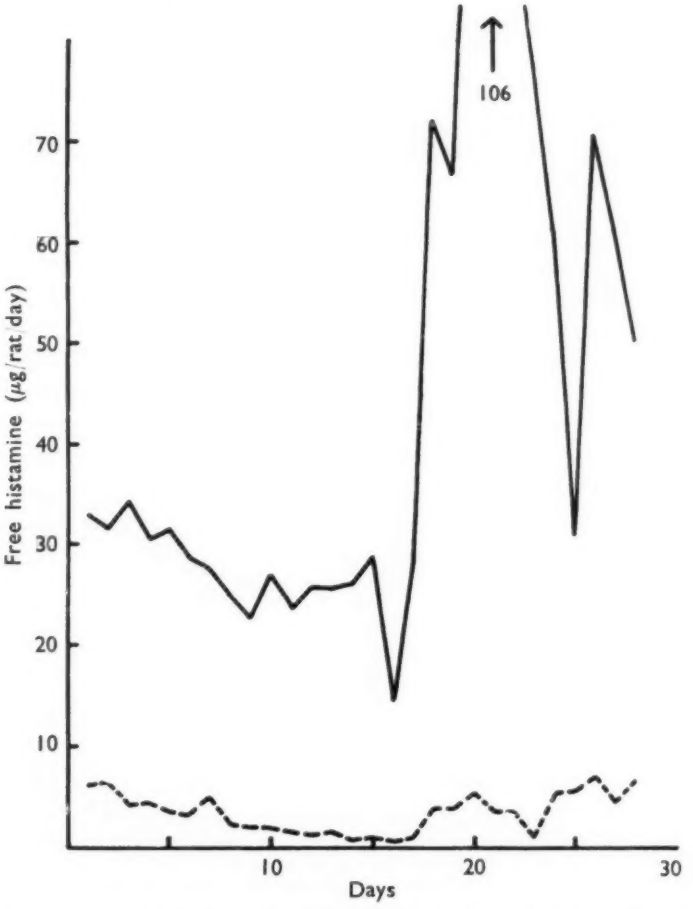

Fig. 6. Effect of compound SKF 525-A and aminoguanidine on the histamine excretion of male (broken line) and female rats (continuous line). All rats received compound SKF 525-A, 1.5 mg/rat/day, 10th to 15th day, and aminoguanidine, 1.0 mg/rat/day, 17th to 22nd day.

conjugation of histamine in rats. Leitch, Debley & Haley (1956) suspected that the difference in histamine excretion was linked with sex, but, in preliminary investigations into this phenomenon, were unable to demonstrate marked changes in histamine excretion after gonadectomy or administration of sex hormones.

The effect of oestrogens is curious, as they themselves produce moderate depression of free histamine excretion in female rats, both when injected and when released endogenously at oestrus. In individual rats this effect is seen to run parallel with a concurrent depression of urine volume at oestrus, and during treatment with oestrogen. It may be therefore that fluctuations of histamine excretion in female rats are simply related to clearance of histamine at varying rates of urine flow.

Association of the sex difference in histamine excretion with the sex hormones is further substantiated by the observations in young growing rats. At weaning the histamine output of both males and females is the same, and the levels only begin to diverge as puberty is approached.

The experiments using acid hydrolysis, though only preliminary, do give some indication of the nature of the conjugation of histamine in male rats. When male rat urine is hydrolysed, about one third of the free histamine excreted by females, or by males after castration or oestrogen treatment, is recovered. Since acetylhistamine is the only known conjugated compound of histamine hydrolysable by Code's method, it would seem that at least one-third of the histamine conjugated by male rats is acetylhistamine. This estimate may well be conservative since as much as 20% of the histamine may be lost during the hydrolysis and subsequent procedure, as indicated by the low "recoveries" of histamine after hydrolysis in some of the urines in which most of the histamine was in the free state. Westling (1958), on the other hand, found only 2 to 4% of acetylhistamine, most of the conjugated histamine being methylhistamine and imidazoleacetic acid. It should be emphasized, though, that the histamine measured in the present work was entirely endogenous, whereas Westling was measuring the excretion of injected histamine and its conjugates.

Further information, though negative, on the nature of the histamine conjugation was provided by the experiments with enzyme inhibitors. Since the effect of aminoguanidine was to increase the free histamine output of both males and females in the same proportion, its action must be entirely by blocking the oxidation of free histamine in both sexes and not by affecting the conjugation of histamine in the males. Compound SKF 525-A has been shown by Cooper, Axelrod & Brodie (1954) to inhibit many detoxication processes including sidechain oxidations, dealkylations, deaminations and ether cleavages, all of which take place in the liver microsomes and require oxygen and reduced triphosphopyridine nucleotide. The observation that compound SKF 525-A has no effect whatever on histamine excretion in either male or female rats eliminates these biochemical mechanisms from participation in the disappearance of free histamine in male rats and possibly also the liver as the site of such activity. Further investigation is therefore necessary to establish the site of histamine conjugation in male rats, to isolate the reaction *in vitro*, and then to study the way in which the mechanisms are influenced by androgens.

The author is indebted to Smith, Kline & French Laboratories for supplying compound SKF 525-A, to May & Baker for aminoguanidine bicarbonate, and to W. Cooper and A. O'Neill for technical assistance.

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THIOXANTHINES WITH POTENT BRONCHODILATOR AND CORONARY DILATOR PROPERTIES

BY

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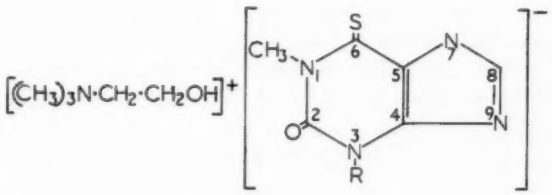
(*Received October* 1, 1960)

Some of the pharmacological properties of two new compounds, choline 6-thiotheophyllinate and the choline salt of 3-isobutyl-1-methyl-6-thioxanthine (M&B 5924), are described. Both these 6-thioxanthines are structurally related to theophylline and pharmacologically they are very similar to that compound, the main differences in their actions being essentially quantitative. They were more potent than choline theophyllinate as bronchodilators on the isolated guinea-pig tracheal ring preparation and as coronary dilators on the dog heart–lung preparation. Choline 6-thiotheophyllinate was about as effective as its oxygen analogue in protecting guinea-pigs against the lethal effects of a bronchoconstrictor aerosol; M&B 5924 was more effective in this respect, but the relative bronchodilator activity was much less than on the isolated preparation. Both thioxanthines were less potent than choline theophyllinate as diuretics. One outstanding qualitative difference in their properties was in their effect on the voluntary motor activity of mice; choline theophyllinate in low doses was stimulant whereas the thioxanthines were either inactive at low doses or sedative at higher doses. In dogs, on the other hand, there were indications that M&B 5924 had a stimulant action.

In the course of testing some new soluble theophylline derivatives, our colleague Mrs. S. Brazier discovered that the choline salt of 6-thiotheophylline (I, R = $-CH_3$) was about four times as active as its oxygen analogue in relaxing the bronchial muscle of the isolated guinea-pig tracheal ring preparation. 6-Thiotheophylline was also found to be a more potent coronary dilator than theophylline. As a result of these observations, other thioxanthines were synthesized and tested pharmaco-



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logically in the hope of finding a compound which was highly active as a bronchodilator or as a coronary dilator, and which was well tolerated and absorbed when given orally. The present paper describes some of the pharmacological properties of such a compound, the choline salt of 3-isobutyl-1-methyl-6-thioxanthine, or M&B 5924 [I, R = $-CH_2.CH(CH_3)_2$].

METHODS

Bronchodilator activity

Isolated guinea-pig tracheal ring preparation. The activity of the compounds as bronchodilators was estimated *in vitro* on the isolated guinea-pig tracheal ring preparation, set up as described by Castillo & de Beer (1947). Changes in muscle tone were recorded on a kymograph by means of a suitably weighted frontal-writing lever. In preparations that rapidly lost tone and did not contract again when the dilator substance was removed from the bath, a low concentration of histamine (10^{-7} to 10^{-6} g/ml. of acid phosphate) was added to restore the tone to its original level. In some experiments, the effect of adding the dilator substance at the height of a histamine-induced contraction was observed. The presence of histamine did not appear to affect the quantitative assay.

Protection against bronchoconstrictor aerosols. In vivo bronchodilator activity was determined by observing whether the compounds would protect guinea-pigs from the bronchospasm induced by exposure to an acetylcholine or histamine aerosol. The guinea-pigs were placed in an anaesthetic box and aerosols were introduced by means of a compressor and a Collison inhaler. Guinea-pigs exposed to a bronchoconstrictor aerosol in this way behaved in a very characteristic manner and showed progressive signs of difficulty in breathing, leading to convulsions and finally death. With little experience the time until signs appeared of a convulsion being imminent (defined by Herxheimer (1952) as the pre-convulsion time) could be judged with considerable accuracy merely by observation of the breathing and behaviour of the guinea-pig. This pre-convulsion time was remarkably constant, if the guinea-pigs were not exposed too frequently. The protection was assessed quantitatively in a similar way to that described by Herxheimer & Rosa (1953). The percentage protection for each guinea-pig was calculated as $(1-\frac{T_1}{T_2})\times 100$, where T_1 is the mean of the control pre-convulsion times two days before and two days after the administration of bronchodilator and T_2 is the test pre-convulsion time determined after administration of the bronchodilator substance. Initially the protective effect of choline theophyllinate and choline thiotheophyllinate was investigated in 8 guinea-pigs each, exposing them 20 min after intraperitoneal injection to either a 2% histamine or 4% acetylcholine aerosol.

Experiments were later performed to see whether M&B 5924 and choline theophyllinate when given orally protected guinea-pigs and for approximately how long such protection lasted. Thirty-two guinea-pigs, deprived of food overnight, were next morning given the compounds by stomach tube, and subjected to a 4% acetylcholine aerosol in groups of 4, at 8 time intervals ranging from 0.5 to 6 hr. The degree of protection obtained by pretreatment with each compound was assessed in the way already described.

Protective effect in the anaphylactic microshock of the guinea-pig. The method used has been described by Herxheimer (1952). Guinea-pigs were sensitized by injection of egg albumen (0.7 ml. of a 5% solution intramuscularly) and 3 weeks later exposed to a 5% egg albumen aerosol. Sensitized animals showed typical symptoms of dyspnoea, whereas normal animals were unaffected. The protective effect of M&B 5924 was assessed by dosing the guinea-pigs orally with M&B 5924 1 hr before exposure to the antigen aerosol.

Microscopic examination of lung sections. The method used was that of Sollman & Gilbert (1937). A rabbit was killed, and the lungs and trachea were excised and filled with warm Ringer solution containing 10% gelatin. The gelatin was allowed to harden in the refrigerator and thin sections of lung were then cut by hand, pinned to a ring of cork and placed in a Petri dish containing Ringer solution at 37° C. After 30 min the gelatin had melted and the preparation had usually recovered from trauma. If the sections of lung contained a nearly transverse section of a bronchus or bronchiole it was possible to observe constriction of the bronchial musculature by addition of acetylcholine or histamine, and relaxation by addition of adrenaline or a theophylline compound. No attempt was made in these experiments to observe more than gross changes and the observer was always aware which drug was being applied to the tissue.

Antihistamine and anti-acetylcholine action on guinea-pig ileum

A piece of ileum was suspended in a 50 ml. bath containing Tyrode solution at 37° C and the tissue was oxygenated with a mixture of 95% O_2 and 5% CO_2. The composition of the Tyrode solution was Na^+ 149 mM, K^+ 2.7 mM, Ca^{++} 1.8 mM, Mg^{++} 1.0 mM, Cl^- 145 mM, $H_2PO_4^-$ 0.4 mM, HCO_3^- 12 mM and dextrose 0.1% (w/v). pA_2 M&B 5924-histamine and pA_2 M&B 5924-acetylcholine were determined with the antagonist in the bath for 14 min as described by Schild (1947). Choline theophyllinate and choline 6-thiotheophyllinate were tested only qualitatively for antihistamine and anti-acetylcholine action.

Cardiovascular studies

Effects on blood pressure. Cats and dogs were anaesthetized with chloralose (80 mg/kg). Blood pressure was recorded from a carotid artery and injections were made into a jugular vein.

Effects on the coronary vessels of the dog heart–lung preparation. The heart–lung preparation of the dog, with a Morawitz cannula in the coronary sinus, was used for measuring effects on coronary blood flow. The preparation was made as described by Burn, Vaughan Williams & Walker (1955). The venous inflow on the right side of the heart was maintained at a constant head of pressure by means of an overflow reservoir, and the excess blood was collected and returned to the reservoir by a Dale-Schuster pump. Changes in the flow of blood from the coronary sinus were recorded by the method of Stephenson (1949) and this coronary blood was then returned to the venous reservoir. The preparation was supplied with oxygen from a partially filled Douglas bag: the Douglas bag was connected to an oxygen cylinder via a flowmeter and the flow adjusted so that oxygen input exactly balanced oxygen consumption. These elaborations were made since changes in oxygen tension and venous pressure are known to affect coronary blood flow (Anrep, 1926). Injections were made into the rubber tubing attached to the cannula in the superior vena cava.

Effects on the vessels of the dog hind leg perfused with heparinized blood. The preparation was made as described by Burn and Rand (1958). Arterial resistance and venous outflow were recorded, the latter by means of a Stephenson recorder (1949).

Effects on the rate and amplitude of the isolated perfused rabbit heart and isolated rabbit atria. The heart was removed from a freshly killed rabbit and perfused with a modified Locke solution (McEwen, 1956) at 32° C by the Langendorff method. The composition of this solution was Na^+ 156 mM, K^+ 5.6 mM, Ca^{++} 2.2 mM, Cl^- 140 mM, $H_2PO_4^-$ 1.2 mM, HCO_3^- 25 mM, dextrose 0.2% (w/v) and sucrose 0.45% (w/v), and it was gassed with 95% O_2 and 5% CO_2. The heart was perfused for about 30 min until the rate and amplitude were steady; by turning a tap the perfusion was then continued with a solution containing a known concentration of M&B 5924.

In the experiments on isolated atria, the atria were carefully dissected from ventricular muscle and as much fat and connective tissue as possible removed, particular care being taken to avoid damage to the atria in the pacemaker region. They were suspended in a 50 ml. bath containing oxygenated double-dextrose Locke solution at 29° C. The composition of the Locke solution was Na^+ 160 mM, K^+ 5.6 mM, Ca^{++} 2.2 mM, Cl^- 164 mM, HCO_3^- 6.0 mM and dextrose 0.2% (w/v), and it was gassed with 100% O_2. Contractions were recorded by a straw lever writing on a smoked drum.

Diuretic activity

In conscious rats. Eight groups of 4 male rats, each weighing approximately 200 g, were used. They were deprived of food overnight and the following morning were given 25 ml./kg of 0.9% saline by stomach tube. Initially, all 8 groups were given saline alone and served as controls; 2 days later they were given orally the test compound dissolved in the appropriate quantity of saline. The animals in each group were then placed in a metabolism cage and the urine was collected and measured at hourly intervals in a graduated centrifuge tube for 5 and in some cases 7 hr. During the test period the rats were left without food and water.

The total volume of urine excreted by each group (V_1) was measured and the % urinary excretion (U) as defined by Lipschitz, Hadidian & Kerpscar (1943) was calculated for each group. If V_0 was the volume of saline originally given to each group, then $U = \frac{V_1}{V_0} \times 100$. There was considerable variation in U among the 8 groups, though the mean value for any given treatment was remarkably constant. The diuretic effects of choline theophyllinate, choline 6-thiotheophyllinate and M&B 5924 were investigated at only one dose, namely, 20 mg/kg, as this dose was shown in a preliminary screening experiment to cause optimal diuresis. Urinary sodium and potassium concentrations were determined with a flame photometer and chloride concentrations were determined volumetrically by Volhard's method.

In anaesthetized rabbits. The method used has been described by Burn (1952). Rabbits of either sex were anaesthetized with urethane. The bladder was cannulated and urine flow was measured with a Thorp impulse counter.

Central effects in mice

Effects on the voluntary locomotor activity of mice were determined using the light-box method of Dews (1953). The dose causing a 50% increase or decrease in motor activity was determined.

Acute toxicity in mice

Intravenous and oral LD50 figures in albino mice were determined by the method of de Beer (1945). In the oral toxicity experiments the mice were deprived of food overnight and deaths were recorded for 2 to 3 days.

Effects on cats and dogs

Choline theophyllinate and M&B 5924 were given orally and intravenously to dogs, and orally to cats, to see whether they caused vomiting or other undesirable effects.

RESULTS

Bronchodilator activity

Isolated guinea-pig tracheal ring preparation. Preliminary experiments on choline theophyllinate showed that over a wide range of concentrations the relaxation of the bronchial muscle was linearly related to the log. concentration of theophylline derivative in the bath. For a quantitative assay, it was therefore considered adequate to determine the relaxation produced by only two concentrations of any compound and to assume a linear relationship between relaxation and concentration. The further assumption was made that the dose-response curves of choline theophyllinate and the thioxanthines were parallel and relative activity was calculated in terms of choline theophyllinate = 1, so that the larger the figure

TABLE 1

RELATIVE ACTIVITY OF TWO THIOXANTHINES TO CHOLINE THEOPHYLLINATE (=1) ON THE ISOLATED GUINEA-PIG TRACHEAL RING PREPARATION

Compound	Mean relative bronchodilator activity ± s.e.	Number of experiments
Choline theophyllinate	1	—
Choline 6-thiotheophyllinate	5 ± 0·4	5
M&B 5924	57 ± 6	12

the greater the activity. In assessing relative activity, no account was made of the fact that the mol. wt. of the three compounds were not all the same, but ranged from 283 for choline theophyllinate to 341 for M&B 5924.

The relative activity of choline theophyllinate and M&B 5924 varied considerably in different experiments. A mean of twelve determinations for M&B 5924 and

five determinations for choline 6-thiotheophyllinate (shown in Table 1) showed them to be respectively 57 times and 5 times more active than choline theophyllinate in relaxing the bronchial muscle of the isolated guinea-pig tracheal ring preparation. Part of an experiment which clearly illustrates the potency of M&B 5924 as a bronchodilator is shown in Fig. 1. Here it is seen that M&B 5924 in a concentration of 1.5 μg/ml. produced a relaxation of approximately similar size to that produced by choline theophyllinate in a concentration of 100 μg/ml.

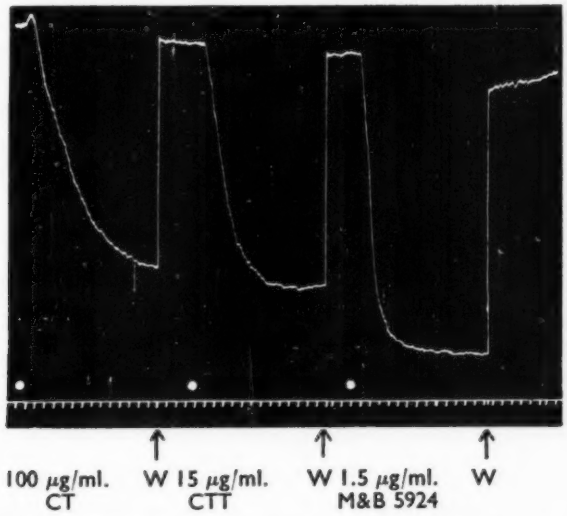

Fig. 1. Isolated guinea-pig tracheal ring preparation showing relaxations of the bronchial muscle of approximately similar size produced by choline theophyllinate (CT), choline 6-thiotheophyllinate (CTT) and M&B 5924. Time marker=60 sec. At W, the bath was washed out several times and the drum stopped until the preparation regained its original tone. Choline theophyllinate sometimes caused a small initial constriction before the dilatation as shown in this figure. This was possibly due to the high concentration of choline. When any of the three compounds were added to the bath, there was always a short latent period before the bronchial muscle relaxed.

Each compound was left in the bath for 15 min, by which time a maximum effect had been attained. Recovery to the original tone after washing out the bath usually took about 30 min and all three compounds appeared to be washed out with equal facility. Recovery time, however, was not measured accurately. An experiment in which choline theophyllinate (CT), choline 6-thiotheophyllinate (CTT) and M&B 5924 were assayed together on a single preparation is shown in Fig. 2.

Protection against bronchoconstrictor aerosols. Control pre-convulsion times were usually between 45 and 90 sec, and if the test pre-convulsion time was greater than 10 min the protection was assumed to be 100%. If the guinea-pigs were accidentally exposed to the bronchoconstrictor aerosol for too long, they convulsed and sometimes died. This happened occasionally with both normal and treated guinea-pigs. If the test pre-convulsion time (T_2) of a guinea-pig that subsequently died had already been determined, then the % protection was

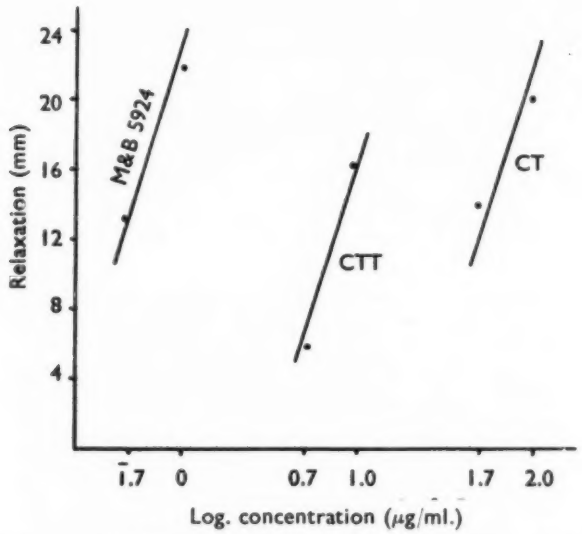

Fig. 2. Quantitative assessment of bronchodilator activity in an experiment on isolated guinea-pig tracheal ring preparation. The mean slope of the three lines was calculated and the three lines were then drawn parallel. Note that the observed relaxations lie very close to the calculated dose/response curves. CT=choline theophyllinate; CTT=choline 6-thiotheophyllinate. Abscissae: log. concentration (μg/ml.). Ordinates: relaxation (mm).

TABLE 2

PROTECTION AGAINST BRONCHOCONSTRICTOR AEROSOLS

Guinea-pigs were injected intraperitoneally with one of the compounds and after 20 min exposed to either a 2% histamine or a 4% acetylcholine aerosol. The mean % protection was calculated as described in the text

Compound	Dose (mg/kg)	Mean % protection ± s.e.	
		Acetylcholine	Histamine
Choline theophyllinate	50	30±6	19±8
	100	61±3	69±8
Choline 6-thiotheophyllinate	50	27±5	26±6
	100	57±3	65±4
Promethazine hydrochloride	0·5	14±8	69±18
	1	23±4	100±0
Atropine sulphate	1	80±14	2±12
	3	100±0	39±3

calculated using only one control pre-convulsion time, and this figure was used in the calculation of the mean % protection of the group.

The results of the experiments on choline theophyllinate and choline 6-thiotheophyllinate, in which protection was assessed 20 min after intraperitoneal administration, are shown in Table 2. The protective effect of choline theophyllinate was almost identical with that of choline 6-thiotheophyllinate against histamine and acetylcholine aerosols. Also included in Table 2 are promethazine and atropine, showing that promethazine gave good protection against histamine aerosols and poor protection against acetylcholine aerosols, whereas the opposite occurred with atropine.

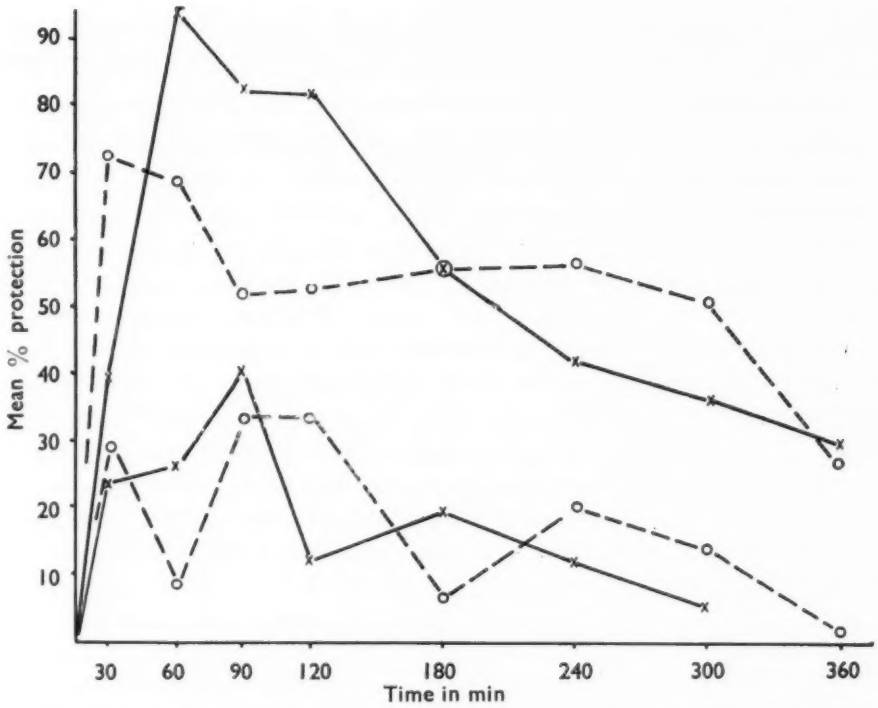

Fig. 3. The protection of choline theophyllinate and M&B 5924 in guinea-pigs exposed to an acetylcholine aerosol. Abscissae: time in min. Ordinates: mean % protection (see text). Each point represents observations on four guinea-pigs. X——X=M&B 5924 (upper curve 100 mg/kg and lower curve 25 mg/kg). O - - - O=choline theophyllinate (upper curve 200 mg/kg and lower curve 50 mg/kg). Note that there was good protection with the high dose of both compounds for the first 2 hr, and still some protection after 6 hr.

M&B 5924 was not tested in this way but was compared with choline theophyllinate for its ability when given by mouth to protect guinea-pigs against acetylcholine aerosols. The results of these experiments are shown graphically in Fig. 3. The lower dose of 50 mg/kg choline theophyllinate corresponds to a dose of 3.5 g in man, which is rather more than is generally given in one day. Although only 4 guinea-pigs were used for each observation, the results clearly show that maximum protection occurred after 1 hr and that there was still some protection at the higher doses after 6 hr. The protection with M&B 5924 (100 mg/kg) appeared to be more complete than with choline theophyllinate (200 mg/kg), but the results were not examined statistically. The high dose of each compound approximated to the maximum single tolerated dose.

Protective effect in the anaphylactic microshock of the guinea-pig. The mean pre-convulsion time of 8 untreated and sensitized guinea-pigs exposed to a 5% aerosol of egg albumen was 61 sec, whereas for 8 other guinea-pigs pretreated with M&B 5924 (100 mg/kg) the pre-convulsion time for all 8 was greater than 4 min. At this dose, the protection was therefore assumed to be complete.

Microscopic examination of lung sections. Qualitative observations only were made, and the concentration of choline theophyllinate required to dilate the

bronchioles by a given amount was as much as 50 times greater than the concentration of M&B 5924 required to cause what was judged to be a similar dilatation.

Antihistamine and anti-acetylcholine action on guinea-pig ileum

The protective effect of choline theophyllinate, choline 6-thiotheophyllinate and M&B 5924 against acetylcholine and histamine aerosols could be explained by an antihistamine or anti-acetylcholine action, although in view of the similar protection against both types of aerosol this did not seem very likely. Experiments were therefore made on the guinea-pig ileum to see if the compounds had a specific antihistamine or anti-acetylcholine action. Large concentrations of all three compounds were required to reduce the stimulant effect of acetylcholine or histamine. Whilst choline theophyllinate was in the bath the effects of acetylcholine and histamine were actually potentiated. After the choline theophyllinate had been washed out of the bath, however, the effects of acetylcholine and histamine were reduced. With choline 6-thiotheophyllinate and M&B 5924 the phase of potentiation was not seen. All three compounds had very little antihistamine and anti-acetylcholine activity. pA_2 values were determined only for M&B 5924, the pA_2 (histamine) being 4.8 and pA_2 (acetylcholine) being 4.2.

Cardiovascular studies

Effects on blood pressure. In the anaesthetized cat and dog, choline theophyllinate, choline 6-thiotheophyllinate and M&B 5924 all caused a transient fall in blood pressure, which in the case of choline theophyllinate was preceded by a transient rise. Doses of the order of 1 mg/kg choline theophyllinate produced a fall in blood pressure similar to that caused by 0.05 to 0.1 mg/kg M&B 5924.

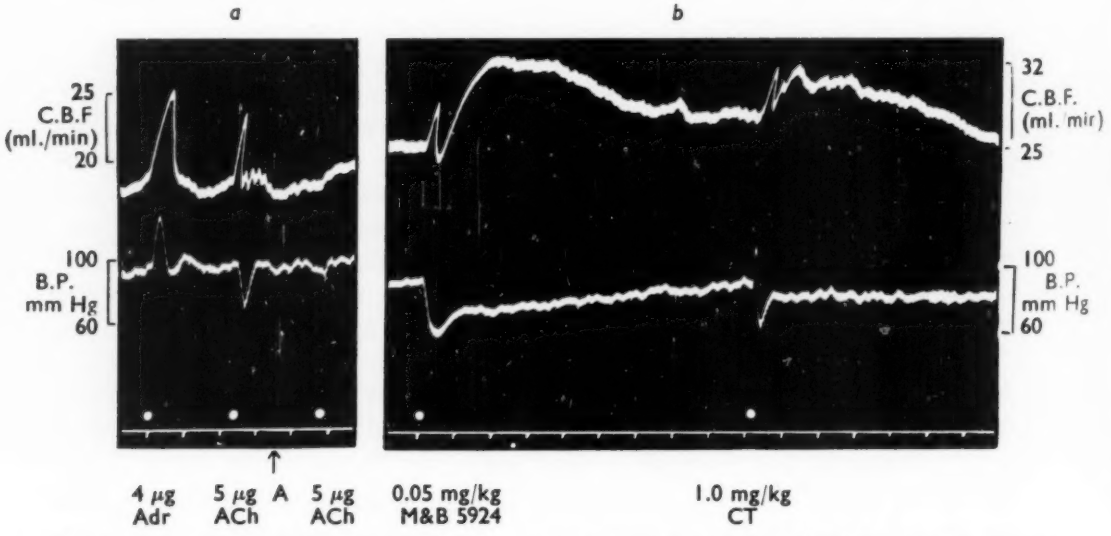

Fig. 4. Dog, 10 kg, chloralose anaesthesia. Upper record: coronary blood flow; lower record: blood pressure in carotid artery. Adr=adrenaline, ACh=acetylcholine, A=1 mg atropine sulphate and CT=choline theophyllinate. Time marker=60 sec. The time interval between (*a*) and (*b*) was 17 min. Note that in spite of the prolonged fall in systemic blood pressure caused by M&B 5924, there was a prolonged increase in coronary blood flow. (A small part of the blood pressure record in (*b*) has been retouched.)

The lower tracing (Fig. 4) shows the effect of 0.05 mg/kg M&B 5924 and 1.0 mg/kg choline theophyllinate on the blood pressure of a dog under chloralose anaesthesia. Choline 6-thiotheophyllinate was more effective than choline theophyllinate in lowering the blood pressure, but less effective than M&B 5924. These falls in blood pressure were only very slightly reduced by atropine and mepyramine.

Effects on the coronary vessels of the dog heart–lung preparation. Coronary blood flow in this paper indicates the blood flowing into the coronary sinus, which Evans and Starling (1913) showed to be about three-fifths of the total blood passing through the coronary arteries.

The coronary blood flow at the start of an experiment was usually about 20 to 30 ml./min. At this stage, the preparation was sensitive to small doses (1 mg and less) of choline theophyllinate, choline 6-thiotheophyllinate and M&B 5924 injected close to the superior vena cava, the volume of circulating blood being approximately one litre. Such doses sometimes caused a 50% increase in coronary flow, without causing any appreciable effect on the heart rate or output. An experiment in which the coronary blood flow was unusually steady is shown in Fig. 5. It is seen that 1.5, 3 and 6 mg of choline 6-thiotheophyllinate caused respectively greater increases in coronary blood flow than 1.5, 3 and 6 mg of choline theophyllinate. Left cardiac output, recorded continuously in this experiment by means of a Stephenson recorder, was not greatly affected by any of these injections and the blood pressure in the lower tracing (=resistance in the artificial circuit) was steady throughout. The coronary blood flow in the dog heart–lung preparation increases slowly but

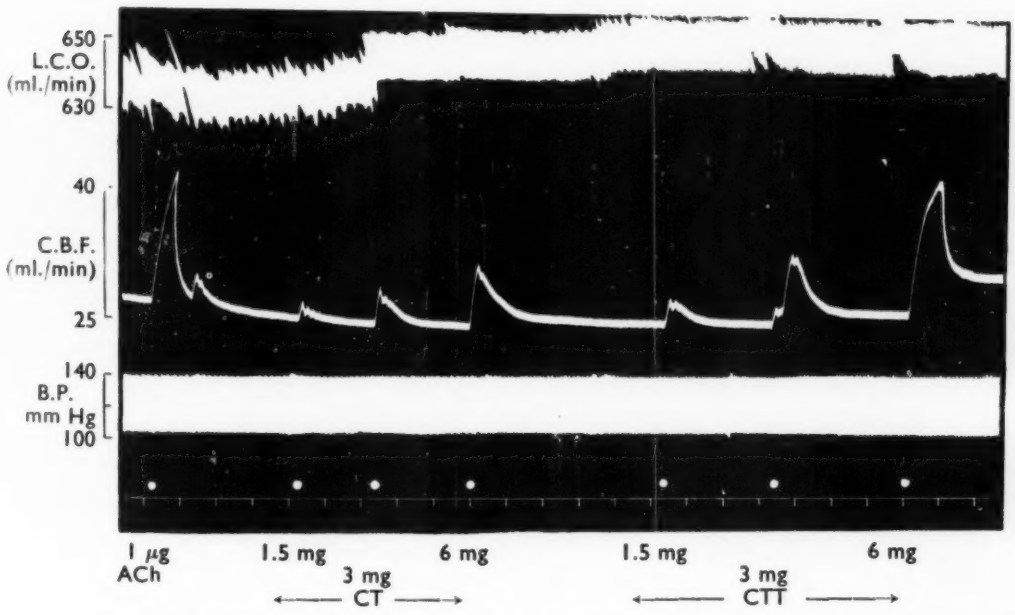

Fig. 5. Dog heart–lung preparation showing left cardiac output (upper record), coronary blood flow (middle record) and blood pressure (lower record). The calibration is shown at the left-hand side of the tracing. ACh=acetylcholine, CT=choline theophyllinate and CTT=choline 6-thiotheophyllinate. All injections were made close to the superior vena cava. Time marker=60 sec.

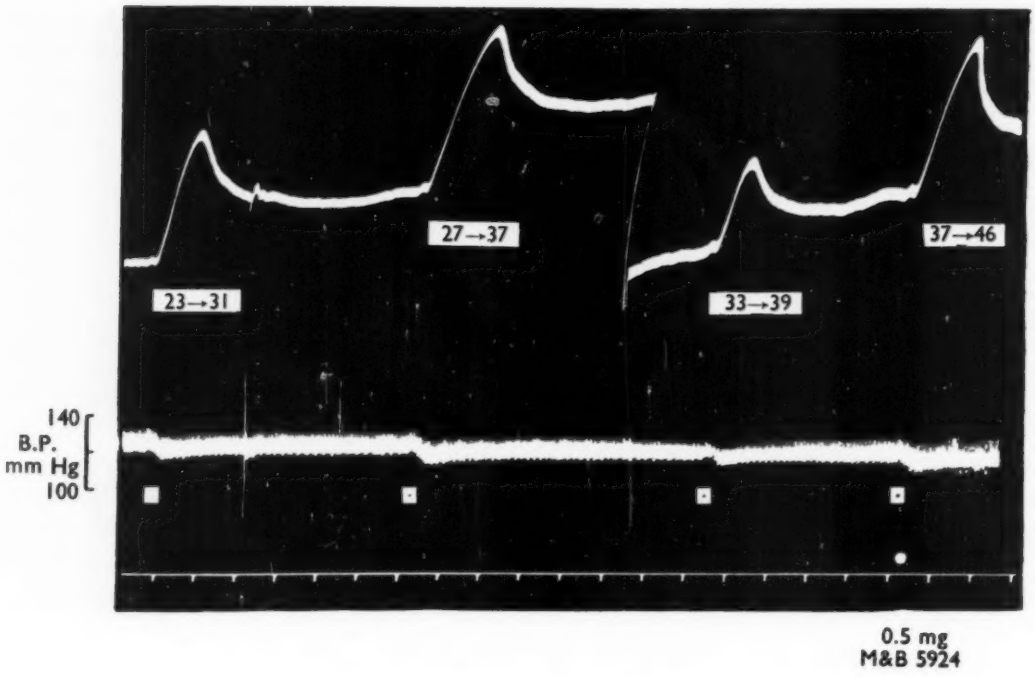

Fig. 6. Record as in Fig. 5, except that left cardiac output was not recorded. At each dot, a xanthine or thioxanthine was injected into the superior vena cava, the fourth compound being M&B 5924 (0.5 mg). Time marker=60 sec. Note that the coronary blood flow gradually increased in this experiment, from 23 ml./min initially to 46 ml./min 20 min later. With each injection there was an increase in coronary blood flow, but only partial recovery to the level before the injection.

spontaneously even when no dilator substances are injected. In the experiment shown in Fig. 5, the coronary blood flow was unusually steady; the more usual course is shown in Fig. 6.

In this experiment, in the course of 20 min, during which two xanthines and two thioxanthines, including M&B 5924, were injected into the superior vena cava, the resting coronary blood flow increased from 23 to 46 ml./min. When the

TABLE 3

CORONARY VASODILATOR ACTIVITY (CDA) ON THE DOG HEART-LUNG PREPARATION AND VASODILATOR ACTIVITY (VDA) ON THE PERFUSED VESSELS OF THE DOG LEG EXPRESSED RELATIVE TO CHOLINE THEOPHYLLINATE=1

Compound	Mean relative CDA	Range of CDA and no. of expts. in parentheses	Mean relative VDA	Range of VDA and no. of expts. in parentheses
Choline theophyllinate	1	—	1	—
Choline 6-thiotheophyllinate	2·5	2–4·5 (6)	2·5	— (1)
M&B 5924	15	8–24 (14)	14	12–16 (3)

coronary blood flow was continually increasing as in this experiment, it was difficult to get more than a rough estimate of coronary dilator activity, because the sensitivity to a given dose of any compound gradually diminished. The coronary dilator activities quoted in Table 3 are therefore only approximations and assessed

from small coronary dilator effects. Doses as high as 10 or 15 mg choline theophyllinate were sometimes required to match the dilator effects of much smaller doses of M&B 5924. Doses of choline theophyllinate as high as this caused noticeable stimulation of the heart, and, whereas the flow reached a peak and then fell to a steady level with small doses of choline theophyllinate, the flow increased and remained high with larger doses. The figures in Table 3 are expressed as the ratios of the dose of choline theophyllinate producing a given increase in coronary blood flow to the dose of test compound producing a similar increase in flow. In some of the experiments on M&B 5924, however, the coronary dilator activity of this compound was not compared directly with choline theophyllinate but with a compound whose activity relative to choline theophyllinate had been determined in previous experiments. The duration of the effect, which was usually short and less than 5 min, is therefore not included in the assessment of coronary dilator activity. The useful " life " of the preparation was about 1 hr ; by this time the coronary blood flow was usually 3 or 4 times greater than at the beginning of the experiment.

Small doses of M&B 5924 (less than 1 mg) had very little effect on the heart rate and no noticeable effect on the output of the left side of the heart. Output was measured by interrupting the flow of blood from the brachiocephalic artery to the reservoir, in the artificial circuit, and collecting the blood in a measuring cylinder for 15 sec. When the dose of M&B 5924 was increased to 5 mg or 10 mg, the heart rate sometimes increased by as much as 20% and the coronary vessels became fully dilated, giving a coronary blood flow of up to 120 ml./min or an increase of about 100 ml./min over the initial value. At this stage, the left cardiac output was invariably less than at the start of the experiment by an amount approximately equal to the increase in coronary blood flow. In spite of these changes in blood flow, M&B 5924 therefore has no effect on the *total* output of the heart.

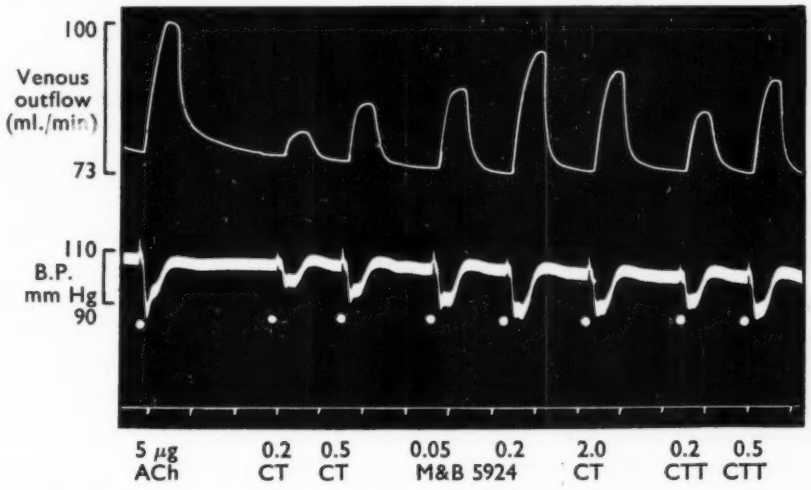

Fig. 7. Perfused vessels of the dog hind leg showing venous outflow (upper record) and arterial resistance=B.P. (lower record). The calibration is shown at the left-hand side of the tracing. ACh=acetylcholine, CT=choline theophyllinate and CTT=choline 6-thiotheophyllinate. All doses were injected intra-arterially, the figures with CT, M&B 5924 and CTT referring to the dose in mg. Time marker=60 sec.

In the anaesthetized animal, M&B 5924 also increased the coronary blood flow (Fig. 4, upper tracing).

Effects on the vessels of the dog hind leg perfused with heparinized blood. The vasodilator activity of choline theophyllinate, choline 6-thiotheophyllinate and M&B 5924 was compared on the vessels of the dog hind leg perfused with heparinized blood. Fig. 7 shows the dilator effects of 0.2 mg, 0.5 mg and 2.0 mg choline theophyllinate (CT), 0.05 mg and 0.2 mg M&B 5924, and 0.2 mg and 0.5 mg choline 6-thiotheophyllinate (CTT). The bottom tracing shows a fall in arterial resistance and the upper tracing an increase in venous outflow, when injections were made into the arterial cannula. The vasodilator activity of the three compounds on this preparation was assessed by bracketing dilator responses caused by different doses, and was almost identical with the coronary dilator activity in the heart–lung preparation (Table 3). Relative activity was calculated in terms of choline theophyllinate = 1.

Effects on the rate and amplitude of the isolated perfused rabbit heart and isolated rabbit atria. When the isolated rabbit heart was perfused with a solution containing 10^{-6} g/ml. M&B 5924, the rate increased by a mean of 22% in 4 different experiments. Effects on the amplitude were variable. Choline theophyllinate and choline 6-thiotheophyllinate were not tested on this preparation, but Schachter, Kimura, Nowarra & Mestern (1954) stated that choline theophyllinate increases the rate and amplitude of the isolated perfused rabbit heart only in concentrations of the order 5×10^{-5} g/ml. On two isolated rabbit atrial preparations, choline theophyllinate (10^{-4} g/ml.) was without any effect apart from a slight slowing; on the other hand, choline 6-thiotheophyllinate (10^{-5} g/ml.) and M&B 5924 (10^{-6} g/ml.) both caused an increase in rate and amplitude.

Diuretic activity

Conscious rats. The results of the experiments are shown in Fig. 8 and Table 4. Chlorothiazide and choline theophyllinate both caused an increase in urine excretion in the first 5 hr, but the diuresis of the rats treated with choline 6-thiotheophyllinate and M&B 5924 closely followed that of the controls. Between 4 and 7 hr, however, choline 6-thiotheophyllinate and M&B 5924 caused a diuresis similar to that caused by choline theophyllinate in the first 4 hr. The effects of these thioxanthines on the excretion of Na^+, K^+, and Cl^- in the first 5 hr are shown in Table 4, together with similar data for chlorothiazide and choline theophyllinate.

In a dose of 20 mg/kg, choline theophyllinate caused a 100% increase in excretion of sodium; choline 6-thiotheophyllinate and M&B 5924 caused increases of 11% and 38% respectively. Chlorothiazide in a dose of 10 mg/kg increased sodium excretion by more than 100%. With all three compounds potassium excretion was increased, and with choline 6-thiotheophyllinate and M&B 5924 chloride excretion was depressed. Although choline 6-thiotheophyllinate and M&B 5924 have been studied in detail only at the one dose level of 20 mg/kg, it is evident that they are of no interest as diuretics.

Anaesthetized rabbits. On this preparation the diuretic activity of the compounds was compared by giving an intravenous infusion in 40 ml. of physiological saline

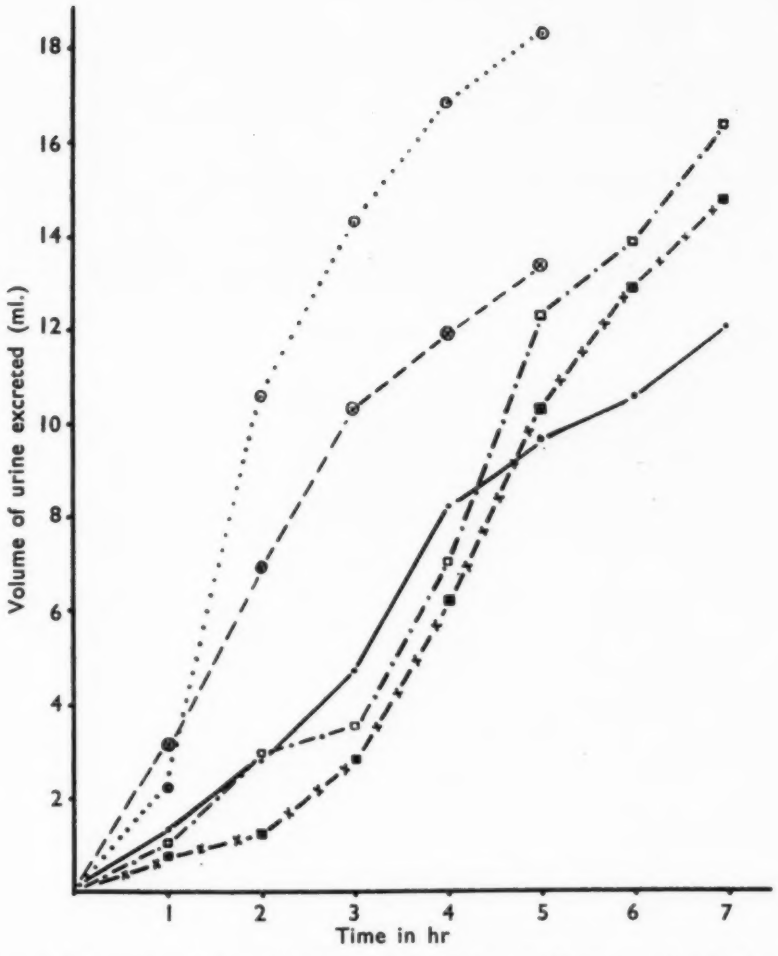

Fig. 8. Diuresis in conscious rats. Abscissae: time in hr. Ordinates: volume of urine excreted in ml. ○ . . . ○ = chlorothiazide ; ⊗ – – – ⊗ = choline theophyllinate ; □– ·–□ = choline 6-thiotheophyllinate ; ■–×–×–■ = M&B 5924 ; and ●——●= controls. Note the powerful and rapid diuretic action of chlorothiazide and the delayed diuresis caused by the thioxanthines.

TABLE 4

URINE AND ELECTROLYTE EXCRETION IN RATS

Compound	Dose (mg/kg)	% urinary excretion	Ionic excretion μeq/100 g/5 hr			*p*H of urine	Na^+/K^+
			Na^+	K^+	Cl^-		
Controls	—	32	89	62	152	6·0	1·44
Choline theophyllinate	20	50	180	102	239	7·0	1·76
Choline 6-thio-theophyllinate	20	40	99	109	96	7·1	0·91
M&B 5924	20	37	123	79	132	6·8	1·56
Chlorothiazide	10	66	201	90	288	6·2	2·24

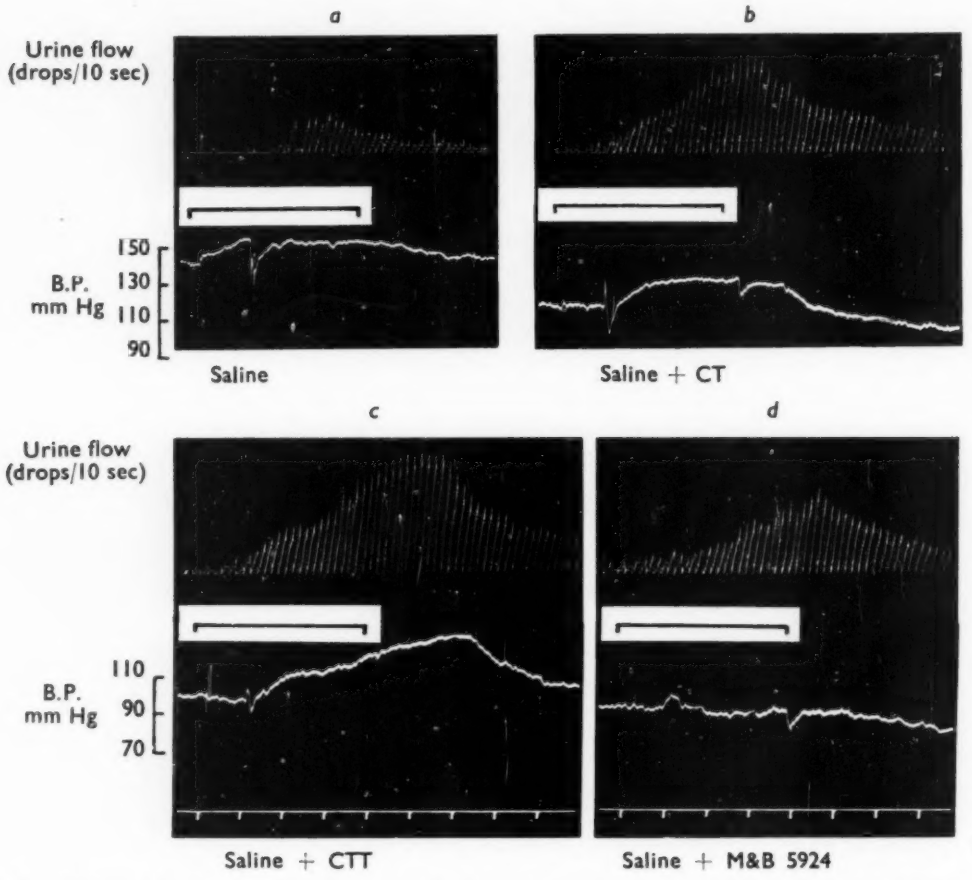

Fig. 9. Diuresis in the anaesthetized rabbit, showing flow of urine in drops recorded by mean of a Thorp impulse counter (upper record) and blood pressure (lower record). In each section an intravenous saline infusion was given over 4 min, as shown by the continuous line; in sections (*b*), (*c*) and (*d*) the saline contained the calculated amount of compound to give a dose of 10 mg/kg. The time interval between the sections was about 20 min. CT=choline theophyllinate; CTT=choline 6-thiotheophyllinate. Time marker=60 sec. Note that the diuresis caused by M&B 5924 was considerably less than that due to choline theophyllinate or choline 6-thiotheophyllinate.

during 4 min. An experiment of this type is shown in Fig. 9. In (*a*) is shown the control diuretic effect of 40 ml. of physiological saline alone. In (*b*) a much larger and prolonged diuresis was caused by 10 mg/kg choline theophyllinate (CT). Choline 6-thiotheophyllinate (CTT) in the same dose was more active than choline theophyllinate (*c*), whereas in (*d*), M&B 5924 caused an effect less than that of choline theophyllinate but greater than that of the control infusion of saline. The sensitivity to any substance and even to the saline alone was liable to change during the experiment, and when the blood pressure fell below 70 mm Hg diuresis sometimes stopped. Effects on the blood pressure were variable though there was usually a slight rise followed by a very slow fall.

In four experiments, choline theophyllinate and choline 6-thiotheophyllinate were found to be about equiactive, whereas M&B 5924 was consistently less active than both these compounds.

Central effects in mice

In doses up to 20 mg/kg choline theophyllinate increased the voluntary locomotor activity of mice. A 50% increase was produced by 12 mg/kg. Choline 6-thiotheophyllinate and M&B 5924, however, in doses from 5 to 80 mg/kg either decreased motor activity or had no effect. A 50% decrease in motor activity was produced by 32 mg/kg choline 6-thiotheophyllinate and by 30 mg/kg M&B 5924.

Acute toxicity in mice

The acute oral and intravenous LD50 figures in mice of choline theophyllinate, choline 6-thiotheophyllinate and M&B 5924 are shown in Table 5. The low values of the ratio B/A indicate that all three compounds were well absorbed from the gastro-intestinal tract. Toxic doses of choline theophyllinate caused intense excitement and convulsions, whereas toxic doses of the thioxanthines caused sedation. Death in all cases was due to respiratory failure.

TABLE 5

ACUTE TOXICITY IN MICE

LD50 figures (mg/kg) in terms of active component and limits ($P=0{\cdot}05$) shown in parentheses

Compound	Intravenous (A)	Oral (B)	Oral/intravenous (B/A)
Choline theophyllinate	74 (70–80)	337 (308–368)	4·5
Choline 6-thiotheophyllinate	39 (34–45)	260 (210–322)	6·7
M&B 5924	51 (47–54)	163 (143–186)	3·2

Effects on cats and dogs

Observations were first made on four dogs, which were given the compounds daily by stomach tube for one week. Choline theophyllinate in a dose as high as 120 mg/kg caused no ill-effects ; M&B 5924, however, caused vomiting and retching at 60 and 80 mg/kg and these effects lasted for about 1 hr after dosing. During this time, the dogs were very quiet, but they had fully recovered within 2 or 3 hr. At 20 mg/kg, M&B 5924 was without effect, and though 40 mg/kg did not make them vomit, this dose obviously caused them distress.

M&B 5924 was given intravenously to 5 dogs in doses up to 3 mg/kg. This dose caused vomiting and retching in 2 of the dogs within 10 min of dosing. Other symptoms included excitation and restlessness, in contrast to the sedation seen in mice. Lower doses were usually without behavioural effects and did not cause vomiting. It was noticed that after an injection of M&B 5924, the dogs sometimes had diarrhoea. Choline theophyllinate, however, in intravenous doses up to 5 mg/kg did not affect 3 other dogs in any way.

The effects of choline theophyllinate and M&B 5924 given to cats in gelatin capsules were also investigated. Both compounds caused salivation (not seen in dogs), but whereas M&B 5924 caused vomiting and retching in similar doses to those tried in dogs, much higher doses of choline theophyllinate were without effect.

DISCUSSION

The diuretic, bronchodilator and coronary dilator properties of theophylline have been known for many years (Goodman & Gilman, 1955). Theophylline itself, however, is an almost insoluble compound which is poorly absorbed and in addition

is highly irritating to the gastric mucosa. Aminophylline, a double salt of theophylline with ethylenediamine, is much more soluble and is a useful and effective drug when given intravenously in the treatment of bronchial and cardiac asthma and Cheyne Stokes respiration, possibly on account of its ethylenediamine content. When given orally, however, aminophylline is relatively poorly absorbed and in sufficiently high doses to produce a therapeutically effective blood level is poorly tolerated. Choline theophyllinate is a theophylline preparation that is claimed to be better tolerated and absorbed than aminophylline after oral administration (Gagliani, de Graff & Kupperman, 1954). Duesel & Fand (1954) have suggested that the reason for the better tolerance of choline theophyllinate is that it is a true chemical compound, stable in the acid secretions of the stomach, whereas aminophylline is merely an addition compound and is unstable under these conditions. The rationale for the better tolerance of choline theophyllinate does not seem very convincing and whether the choline moiety is important seems dubious. Nevertheless, the thioxanthines were tested as their choline salts, since this salt for both compounds could be prepared in a crystalline form which was readily soluble in water and was therefore very suitable for pharmacological study.

In most of their pharmacological properties, choline theophyllinate, choline 6-thiotheophyllinate and M&B 5924 differed only quantitatively. Both the thio-compounds were more active than choline theophyllinate as bronchodilators and coronary dilators, but less active as diuretics. Choline theophyllinate, however, showed stimulant properties in increasing the voluntary motor activity of mice, whereas its thio-analogue and M&B 5924 both decreased motor activity.

In view of the high bronchodilator activity of M&B 5924 on the isolated guinea-pig tracheal ring preparation, the protection this compound gave to guinea-pigs exposed to bronchoconstrictor aerosols was surprisingly low. This was unexpected since the ratio of oral LD50: intravenous LD50 in mice (Table 5) was low, suggesting that oral absorption was good. It is possible, however, that oral absorption in guinea-pigs was not as good as in mice.

The extremely weak antihistamine and anti-acetylcholine action of choline theophyllinate, choline 6-thiotheophyllinate and M&B 5924 on the guinea-pig ileum and the almost identical protection that choline theophyllinate and choline 6-thiotheophyllinate afforded against acetylcholine and histamine aerosols suggested that the protection was due to a genuine bronchodilator action and not due to an anti-acetylcholine or antihistamine action. Aminophylline in these experiments was incidentally found to be very similar in activity to choline theophyllinate. Herxheimer & Rosa (1953) investigated the protective effect of sympathomimetic amines and of aminophylline in the anaphylactic microshock of the guinea-pig. With high doses of aminophylline (100 mg/kg) full protection was achieved, but the sympathomimetic amines, isoprenaline, adrenaline, noradrenaline and ephedrine, all caused toxic symptoms before full protection was achieved. In our experiments, M&B 5924 in the dose of 100 mg/kg also afforded full protection.

The observations on lung sections showed that the dilatation caused by M&B 5924 was not merely confined to the bronchial muscle of the trachea but acted presumably on all the smooth muscle of the bronchial tree. Our experiments indicate that M&B 5924 was more active than choline theophyllinate. Offset

against the increased activity, however, is the possibility that sufficiently high blood levels may not be achieved for a therapeutic effect.

The experiments on the dog heart–lung preparation clearly demonstrated that choline 6-thiotheophyllinate and M&B 5924 were more effective than choline theophyllinate in increasing the flow of blood through the coronary vessels. Under the conditions of the heart–lung experiments, the venous pressure was constant, and, since the blood from the left side of the heart was diverted through a fixed peripheral resistance, generalized vasodilatation was not possible. Coronary dilator effects were observed with small doses of M&B 5924, which had no effect on heart rate, counted for 15 sec with a stop-watch, or cardiac output. It is therefore likely that the increased coronary blood flow was due to a direct action on the coronary vessels. Larger doses (5 to 10 mg) of choline theophyllinate and the thioxanthines, however, caused an increase in heart rate, and with M&B 5924 very large increases in coronary flow. A cardio-accelerator action was also observed on the isolated atrial preparation and was therefore probably due to an effect on the pacemaker.

In the whole animal, the effect on coronary blood flow was complicated by the fall in systemic blood pressure which these compounds caused. Nevertheless, in spite of the relatively prolonged hypotensive action of 0.05 mg/kg M&B 5924 in the dog (Fig. 4), there was also an increase in coronary blood flow. Le Roy & Speer (1940) have reported that various dialkylxanthines are more potent coronary dilators than theophylline in the anaesthetized dog, and Quimby, Aviado & Schmidt (1958) have reported the effects of aminophylline and other xanthines on the pulmonary circulation of the dog. Quimby *et al.* show the chemical structures of xanthines, including some thioxanthines, that caused non-selective vasodilatation and vasoconstriction. These authors state that replacement of oxygen in the 2- or 6- position did not affect dilator action, but it is not clear which thioxanthines were examined. 1,3-Dialkylthioxanthines, however, do not appear to be excluded from their study. We have not studied the effects of M&B 5924 on the pulmonary circulation, but in addition to dilating the coronary vessels it also dilated the vessels of the hind leg of the dog. M&B 5924 is therefore also likely to be a non-selective dilator and it is most likely that the main cause of its hypotensive action is vasodilatation of the peripheral vessels.

By comparison with chlorothiazide, the thioxanthines were relatively ineffective diuretics. When rats were given choline 6-thiotheophyllinate or M&B 5924 orally, the diuresis in the next 5 hr scarcely differed from that of control rats, although a marked diuresis usually occurred between 5 and 8 hr. One possible explanation of this delayed diuresis is that the $\rangle C{=}S$ group is converted in the body to $\rangle C{=}O$.

The only experimental comparison of the central properties of the thioxanthines with those of theophylline has been by observation of the effects on the voluntary locomotor activity of mice. It was interesting to find that both thio-compounds were weakly sedative whereas theophylline was weakly stimulant. One of the objects of this research was to find a compound which would relieve bronchospasm and consequent restlessness in the chronic asthmatic ; a compound with a sedative action as opposed to the stimulant action of theophylline would therefore be an

advantage. In dogs, however, M&B 5924 appeared to have a stimulant rather than a sedative effect. The fact that M&B 5924 caused vomiting in some dogs when given intravenously also suggests a central stimulant action. There is therefore the possibility that, when given orally, M&B 5924 may cause vomiting by a local irritant action in the stomach and after absorption by a central mechanism. The emetic properties of M&B 5924 and related compounds are being studied in more detail.

We are grateful to our colleagues, Drs. R. Slack and K. R. H. Wooldridge, for synthesizing choline 6-thiotheophyllinate and M&B 5924; to Professor J. H. Burn, F.R.S., and Dr. J. M. Walker for demonstrating the heart–lung preparation and insertion of a Morawitz cannula into the coronary sinus; and to Messrs. G. T. Langston, M. A. Ogilvie and A. C. Rasmussen for excellent technical assistance.

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THE EFFECT OF RESERPINE ON THE PRESSOR RESPONSE TO INJECTION OF 2-HALOGENOETHYLAMINES

BY

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(*Received October* 4, 1960)

Dibenamine-like compounds sometimes caused an increase in blood pressure when injected intravenously in mammals. This response varied with species, with the preparation, and with the structure of the compound. The response became smaller after repeated injections. In spinal atropinized rats injected with hexamethonium 5 mg/kg this pressor effect produced by injection of the adrenaline antagonist, compound AT3 [ethylfluoren-9-yl(2-iodoethyl)amine hydriodide], was reduced by prior treatment for five days with 1 mg/kg reserpine. Acute adrenalectomy also reduced this effect of the adrenaline antagonist; reserpine plus adrenalectomy abolished it. Subsequent injection of adrenaline and noradrenaline restored it.

It is well known that compounds like phenoxybenzamine [benzyl-(2-chlorethyl)(1-methyl-2-phenoxyethyl)amine] antagonize the pressor response to injected catechol amines and lower the blood pressure for prolonged periods. Less attention has been paid to the acute pressor response which may be caused by intravenous injection of the 2-halogenoethylamine itself. A study of this phenomenon may reveal an aspect of the activity of these compounds which is an essential preliminary to their antagonistic effect.

METHODS

Demonstration of the pressor action. Carotid blood pressure was recorded manometrically from the following preparations: (*a*) dogs anaesthetized with 40 mg/kg pentobarbitone sodium intravenously and injected with 5 mg of atropine sulphate subcutaneously, (*b*) atropinized spinal cats, (*c*) atropinized spinal rats with or without injection of 5 mg/kg of hexamethonium hydrobromide and with and without acute bilateral adrenalectomy. Three 2-halogenoethylamines were tested, (2-bromoethyl)ethyl(naphth-1-ylmethyl)amine hydrobromide (SY28 or J11, Graham & Lewis, 1953), (*p*-chlorobenzyl)(2-chloroethyl)ethylamine hydrochloride (JFA8) and ethylfluoren-9-yl(2-iodoethyl)amine hydriodide (AT3, Graham, 1960). In all cases the compounds were dissolved from the crystalline state in minimal volumes of acetone, diluted with neutral saline, and injected intravenously at once in a fixed volume in a dosage of 10 μg/kg of compound AT3 to 1.0 mg/kg of compound J11. For rats this was 0.1 ml. per injection, washed in with 0.2 ml. of saline. Control observations were always made with the same volume of solvent similarly injected. The injections were repeated three times at intervals of 15 min with or without the interspersion of injections of 4 μg/kg adrenaline or noradrenaline (calculated as base) in cats and dogs, 1 μg/kg in rats.

Isolated preparations. Doses of 1 μg to 1 mg of each of the three compounds were injected into the fluid perfusing standard preparations of (*a*) isolated rabbit hearts (each drug was tested on three preparations) and isolated perfused hearts of young cats and (*b*) perfused vessels of the rabbit ear.

Effect of reserpine on the pressor action. Twenty-five male hooded rats of approximately 300 g were treated with 1 mg atropine, a spinal preparation was made and hexamethonium

hydrobromide 5 mg/kg injected intravenously. The effect on the blood pressure of compound AT3 10 μg/kg in 0.1 ml. of solution was recorded and the injection repeated three times. Ten rats treated in this way were subjected to acute bilateral adrenalectomy at the time of making the spinal preparation. Thirty more were pretreated with reserpine 1 mg/kg daily subcutaneously for five days and 10 of them adrenalectomized at the time of making the spinal preparation.

RESULTS

Pressor action. In anaesthetized atropinized dogs the pressor response to injection of any of the three 2-halogenoalkylamines was more dramatic than in spinal cats or rats, despite the greater sensitivity of the latter preparations to injected adrenaline or noradrenaline. Fig. 1 shows a particularly striking example of the pressor response in a dog to injection of compound JFA8. As the response to injected adrenaline or noradrenaline declined or was reversed, so did the response to injected JFA8 alter.

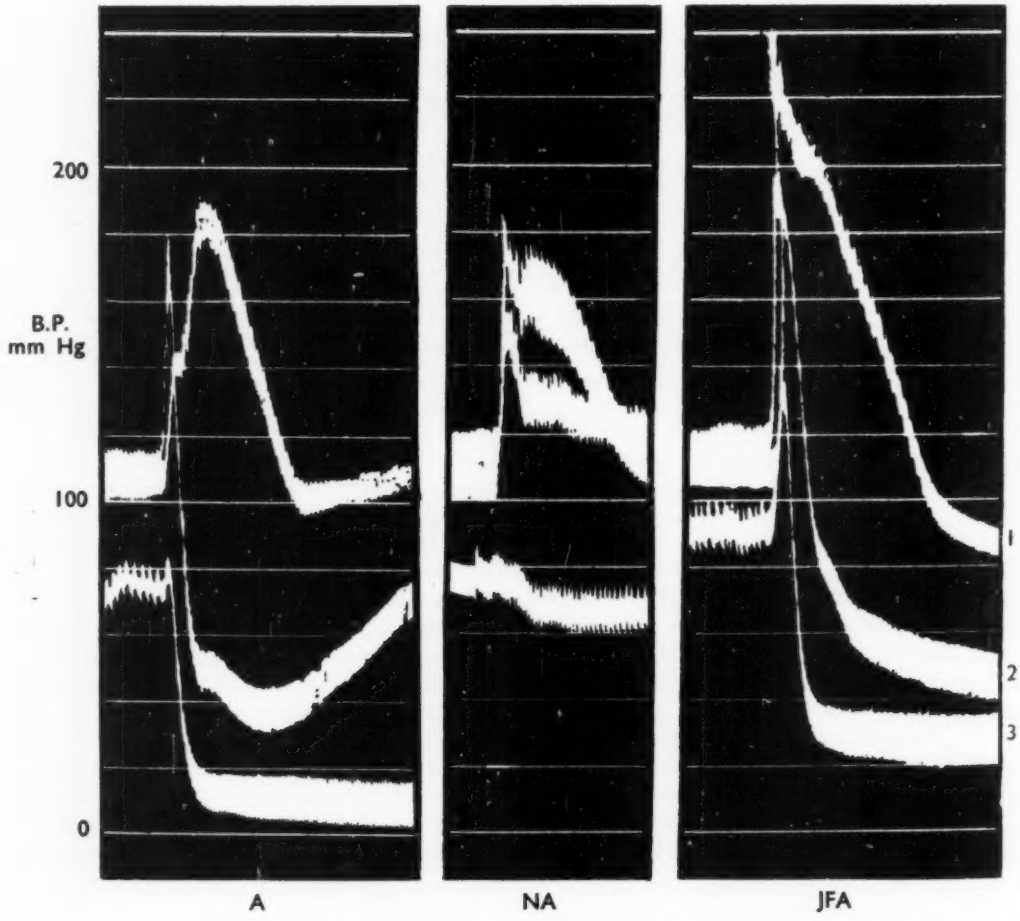

Fig. 1. Dog, 16.3 kg, anaesthetized with pentobarbitone sodium 40 mg/kg intravenously and treated with atropine sulphate 2 mg. Effect on arterial blood pressure of adrenaline (A) 4 μg/kg noradrenaline (N) 2 μg/kg and compound JFA8 (JFA) 1 mg/kg, injected in that order and repeated twice (1,2,3).

In spinal cats and rats this acute pressor effect was variable. In rats the response to a standard dose of compound AT3 (which is the difference between the pressor response in mm Hg to the paired injections of saline and of compound) was slightly greater when hexamethonium has been given ($0.3>P>0.2$). This may be due to the lower initial level of blood pressure following injection of hexamethonium. In 25 such rats the mean response was 4.7 ± 4.9 mm Hg (range 0–16).

Acute bilateral adrenalectomy reduced the response but did not abolish it entirely in rats given hexamethonium, but if adrenalectomy was performed and hexamethonium not administered there was no detectable pressor response.

Repetition of the injections of 2-halogenoalkylamines resulted in a reduction of the pressor response (see Fig. 1) and in some experiments the response was either abolished or reversed. There was an apparent parallelism between the degree of antagonism of the injected catechol amine exerted by the compound and the reaction of the blood pressure to an injection of the compound itself. Thus the response to injection of compound AT3 for the first time in the 25 rats was a pressor response of 4.7 mm Hg, on the fourth injection it was a depressor response of 2.4 ± 3.2 mm Hg.

Action on isolated preparations. In small doses (10 μg of compound AT3 to 100 μg of compound J11) these compounds caused a transient stimulation of the isolated perfused heart of cat and rabbit. In doses 10 times greater they produced a strong and prolonged depression. When administered in small doses (10 μg of compound AT3, 100 μg of compound J11) they also caused a small and transient constriction of the perfused vessels of the rabbit ear.

Effect of reserpine. Pretreatment of rats with reserpine reduced the pressor response to injected compound AT3 to a very low level, and additional adrenalectomy abolished it. This reduction of the response after reserpine was statistically significant (see Table 1). If adrenaline and noradrenaline were injected into

TABLE 1

PRESSOR RESPONSES IN ATROPINIZED SPINAL RATS

The response was measured before and after hexamethonium hydrobromide (C6) 5 mg/kg, after additional treatment with reserpine 1 mg/kg for 5 days and after subsequent injections of adrenaline and noradrenaline (A–NA) 1 μg/kg

Expt. no.	No. of rats	Treatment	Pressor response to AT3	Difference
1	25	Nil	Present	—
2	25	C_6	Greater	$0{\cdot}2>P>0{\cdot}2$
3	20	Reserpine+C_6	Less	$0{\cdot}01>P$
4	20	Reserpine+C_6+A–NA	Greater	$0{\cdot}02>P>0{\cdot}01$

acutely prepared rats pretreated with reserpine before giving the compound AT3, the effect of the reserpine was not apparent and compound AT3 gave a pressor response. If compound AT3 was injected into acutely prepared rats pretreated with reserpine before and again after this restorative injection of catechol amine, the difference in response was very apparent. Fig. 2 illustrates the recovery of the pressor reaction to compound AT3 in this way, and Table 1 records the degree of statistical significance found in the series of trials performed.

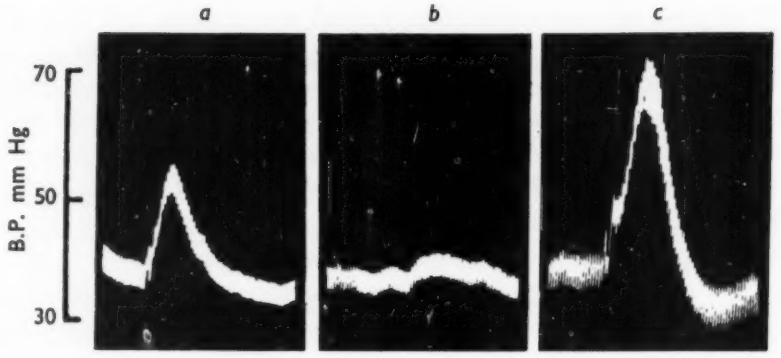

Fig. 2. Pressor responses of spinal atropinized rats to injections of 10 μg/kg of compound AT3 in 0.1 ml. of solvent, washed in with 0.2 ml. of saline. All three preparations received hexamethonium hydrobromide 5 mg/kg. (*a*) Preparation without any other treatment, (*b*) preparation given reserpine 1 mg/kg on 5 successive days, (*c*) preparation given reserpine as in (*b*) and an additional injection of adrenaline and noradrenaline, 1 μg/kg.

DISCUSSION

The transient rise in blood pressure which occurs after injection of 2-halogenoethylamines might be attributed to a central stimulant action affecting the sympathetic nervous system. It may in part be due to a direct cardiac stimulant effect. Millar, Keener & Benfey (1959) have shown that intravenous injection of phenoxybenzamine raises the plasma levels of adrenaline and noradrenaline in dogs and the plasma level of noradrenaline in adrenalectomized dogs, and Benfey, Ledoux & Melville (1959) proved that increased plasma levels of adrenaline and noradrenaline result in an increased urinary excretion of catechol amines. These effects they attributed largely to nervous stimulation, reflex or direct, of adrenal medullary secretion because administration of hexamethonium prevented them.

The three compounds used in the present experiment cause more depression than stimulation of the central nervous system on intravenous injection into mice; the bromo-analogue of compound AT3 is a sedative but is not synergistic in this respect with reserpine (Graham, 1960). The pressor phenomenon is seen in spinal preparations where the influence of central actions is less likely to predominate. The adrenal medulla must be an important source of the pressor effect since adrenalectomy reduces it, but the effect of hexamethonium in the spinal rat is equivocal. These results suggest a direct release of pressor amines from the adrenal medulla while nervous influence is reduced by the ganglion blocker. Brown & Gillespie (1957) and Brown, Davies & Gillespie (1958) have shown that administration of phenoxybenzamine causes a greater release of amine from a tissue when the sympathetic nerve supply is stimulated than takes place without the influence of this 2-halogenoethylamine, while Schapiro (1958) demonstrated an increased urinary excretion of noradrenaline and a loss of catechol amines from the heart, adrenals and spleen after giving this compound.

Reserpine is known to disperse noradrenaline from various tissues including artery walls (Burn & Rand, 1958). The present experiments indicate that the transient pressor response in rats to 2-halogenoethylamine is reduced by treatment with reserpine and restored by infusion of catechol amines.

It may be surmised that 2-halogenoethylamines release catechol amines from tissue stores, including the adrenal medulla, by direct action. This might account for a number of their actions, such as the initial pressor response which may follow injection, the initial increase in sensitivity to injected adrenaline (Graham & Lewis, 1953), the rise in plasma levels of adrenaline and noradrenaline, increased urinary excretion and the fall in tissue content of catechol amines and the reduced excitability of cardiac muscle in response to hydrocarbon anaesthetics.

Presumably the increased catechol amine excreted after treatment with phenoxybenzamine has not been subjected to *O*-methylation.

The relation between possible release of pressor amines by these compounds from the tissue store, occupation of the receptor and the normal inactivation of catechol amine is close.

I am indebted to Mr. G. Rowland for much technical assistance and to Professor N. B. Chapman, Hull, for compounds.

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THE ACTIONS OF AMYLOBARBITONE SODIUM, BUTOBARBITONE AND QUINALBARBITONE SODIUM UPON INSOMNIA AND NOCTURNAL RESTLESSNESS COMPARED IN PSYCHIATRIC PATIENTS

BY

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(*Received October 5*, 1960)

Amylobarbitone sodium, butobarbitone, quinalbarbitone sodium, and a placebo were compared in a controlled, double-blind trial in psychiatric patients with insomnia. An apparatus recording movement in bed is described, with sleep assessments by night nurses and patients. All three barbiturates significantly reduced movement and gave significantly longer, less broken and sounder sleep, without increased incidence of "hang-over." There was no follow-on effect from night to night. With effective doses there was no significant difference between these barbiturates in onset and duration of hypnotic action, although usually accepted as either short or intermediate acting.

The selection of a barbiturate from the large number available is, in clinical practice, often arbitrary or dependent upon local fashion. Those placed in the short-acting group are more often used with patients who cannot readily get to sleep, whilst the intermediate-acting barbiturates are prescribed for patients wakeful in the latter part of the night. Recently, the duration of action, by which these barbiturates are grouped, has been questioned. In order to evaluate the clinical hypnotic action of the widely-used amylobarbitone sodium it was compared with quinalbarbitone sodium and butobarbitone, accepted as having a short and an intermediate action respectively.

METHODS

Patients. The patients studied slept in a ward of 10 moderately disturbed men with a night nurse always present. They had insomnia due to their mental illness and would normally have received a hypnotic drug. The diagnoses in the 16 patients completing the trial were depression (9), schizophrenia (2), anxiety state (1), hypomania (1), alcohol addiction (1), personality disorder with depressive features (1) and hypochondriacal features (1). During the period of study no other medicinal or physical treatments, for example, electro-convulsive therapy, were given. For nine patients such treatment was indicated before the end of the trial period and so the trial was stopped and the records discarded, the next suitable patient being substituted. The patients had their last meal of the day by 6 p.m., with cocoa and biscuits between 8 and 9 p.m. At 10 p.m. they got into bed and took the trial drug contained in two gelatin capsules. On the first three nights of recording the capsules contained 0.2 g (3 grains) of pentobarbitone sodium. This preliminary period enabled the patient to become familiar with the ward environment and

the procedure of the trial; the records of this period were discarded. Considerable spontaneous improvement occurred in 10 patients during these three days and they were rejected at that point from the fully recorded trial. The final 16 subjects had their sleep recorded for eight nights each. The capsules containing the three barbiturates and the placebo could not be distinguished and their contents were not revealed to the patients and nurses assessing the results. The drugs were given on successive nights according to an 8×8 Latin square, the patients acting as their own controls. This square was designed (Williams, 1949) to enable one to calculate if there were a follow-on effect from any one night to another. The patients were allotted at random to the rows of the square and the square replicated for the second group of eight patients. Each patient had, in a different sequence, amylobarbitone sodium 0.2 g and 0.1 g, quinalbarbitone sodium 0.2 g and 0.1 g, butobarbitone 0.2 g and 0.1 g and,

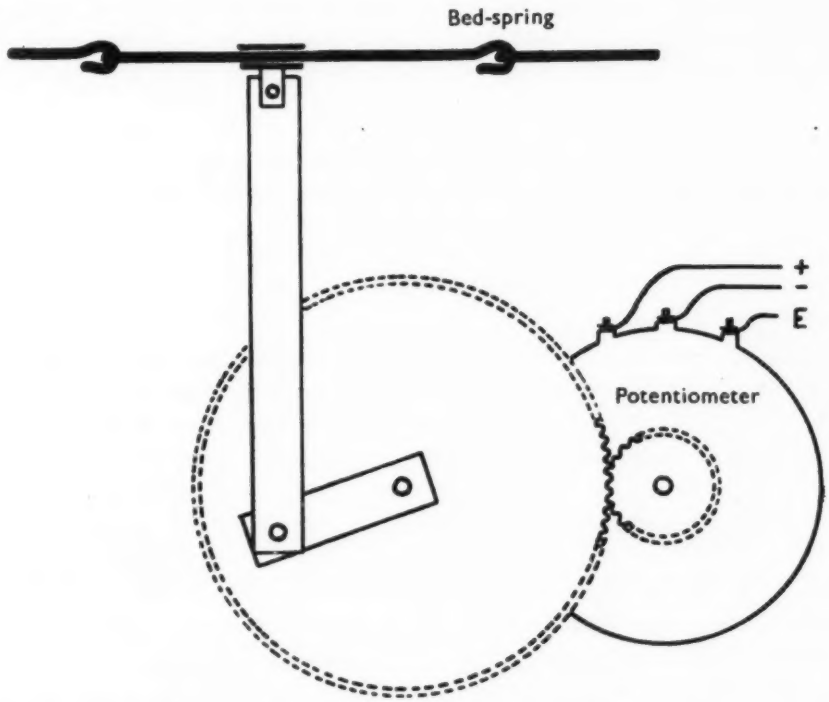

Fig. 1. Diagram of the apparatus which was attached to the bed so that the movements of the patient, which displaced the bed-spring, rotated the potentiometer.

twice, a placebo, lactose, as a comparison. The effects of these drugs were recorded in three ways: firstly, an electronic device to measure movement in bed; secondly, the night nurses' estimate of sleep or wakefulness; and, thirdly, some subjective estimates by the patient.

Movement record. The apparatus used to record the motility of the patient has been slightly modified from that described by Cox & Marley (1959) and used to assess the hypnotic value of two drugs (Hinton & Marley, 1959). Two upright rods were attached to the centre of the bed-spring under the patient's hips and shoulders and they were displaced vertically when the patient moved (see Fig. 1). By means of a crank these vertical movements were modified to rotate a potentiometer, the rotary excursion being increased through two gear-wheels. The voltage differences produced by turning the potentiometer were fed into the circuit described by Cox & Marley (1959) and amplified to provide enough power to work a counter and a pen recorder, which were kept in a side-ward. The counter gave the total motility score and provided a check when counting the score recorded on the paper, which

moved through the pen recorder at 1 inch/2 min throughout the night. A small movement of the trunk usually scored 1 or 2, whereas a vigorous complete change of position would score 6 or 8.

Nurses' record. The night nurse recorded every half-hour if the patient appeared asleep or awake. She also noted the time of getting to sleep or waking and any periods of wakefulness during the night.

Patients' record. The following morning the patient was asked to mark on a simple questionnaire his own estimate of the quality of his sleep (sound, fair, poor, or very poor), whether or not he had a "hang-over," and, if he did, how it affected him.

RESULTS

Movement record. The movements scored by the pen recorder were counted and subjected to analysis of variance. Table 1 gives an example of the method applied to the total motility score for the night. Table 2 shows the application

TABLE 1
TOTAL MOTILITY SCORE FOR THE NIGHT
Analysis of variance showing highly significant difference between drugs and between patients

	Degrees of freedom	Sums of squares	Mean square	Variance ratio	Probability
Between squares	1	3,528	3,528	6·5	<0·05
Between occasions	14	4,446	318	0·6	—
Between patients	14	90,444	6,460	11·9	<0·001
Between drugs	7	20,607	2,994	5·4	<0·001
Interaction of drugs and squares	7	2,326	332	0·6	—
Residual	84	45,690	544		
Total	127	167,041			

TABLE 2
MEAN TOTAL MOTILITY SCORES FOR THE NIGHT
t test applied to comparison of barbiturates separately with each placebo night

	0·2 g			0·1 g			Placebo	
	Buto-barb.	Quinal-barb. sod.	Amylo-barb. sod.	Buto-barb.	Quinal-barb. sod.	Amylo-barb. sod.		
Mean score	18·5	26·9	29·8	49·1	38·8	36·9	52·7	56·9
t	4·15	3·13	2·7	0·44	1·69	1·93	—	—
Probability	<0·01	<0·01	<0·01	>0·05	>0·05	>0·05	—	—

of the *t* test to the mean total motility score for each drug as compared with the placebo nights; it was thought to be a stricter test of significance to compare the barbiturate with both placebo nights separately. If it was different from only one placebo night it has not been considered further. Using the methods outlined by Williams (1949) possible carry-over or residual effects of one drug on another were tested in the case of the total motility score for the night, but, as this gave no indication that such effects were present, it was not considered necessary to carry out this more elaborate analysis for the other scores.

The motility scores after the first hr, the first 2 hr and the first 4 hr were compared between the barbiturates and the placebos to see how long it took for a significant difference to appear. Table 3 shows that after 1 hr 0.2 g butobarbi-

TABLE 3

MEAN SCORES OF MOVEMENT; OF ONSET, DURATION, AND INTERRUPTIONS OF SLEEP; AND OF QUALITY OF SLEEP AND "HANG-OVER"

Over-all significance of difference between drugs assessed by analysis of variance (probability column): difference between pairs of means by *t* test (*=$P<0.05$, †=$P<0.01$ when compared with placebos)

	0·2 g			0·1 g					
	Buto-barb.	Quinal-barb. sod.	Amylo-barb. sod.	Buto-barb.	Quinal-barb. sod.	Amylo-barb. sod.	Placebo		Proba-bility
Movement score									
After 1st hr	3·5*	7·0	8·5	10·6	8·6	9·8	11·0	13·5	<0·05
After 1st 2 hr	5·4†	7·9*	11·4	15·9	11·6	13·7	14·9	18·8	<0·01
After 1st 4 hr	8·1†	12·5†	17·6*	31·1	19·6	18·0*	30·8	29·4	<0·001
After 2nd 4 hr	10·4*	14·4	12·3*	18·1	14·3	18·9	21·9	27·5	<0·01
After 8 hr	18·5†	26·9†	29·8†	49·1	38·8	36·9	52·7	56·9	<0·001
Total sleep in hours	7·09*	7·06*	7·06*	6·22	6·63	6·28	5·66	5·88	<0·05
Time to get to sleep, in min	35·6	29·1	30·3	52·5	41·3	51·6	57·5	41·3	>0·05
Breaks in sleep	0·56*	0·44*	0·69	0·75	1·19	1·06	1·75	1·31	<0·01
Quality of sleep (sound=1 to 4)	1·81	1·56†	1·69*	1·94	1·88	2·19	2·31	2·81	<0·001
"Hang-over" (total incidence)	4	3	3	3	2	3	2	4	—

tone had significantly reduced restlessness as compared with both placebo nights. After 2 hr quinalbarbitone 0.2 g also showed statistically significant effect. After 4 hr all three barbiturates in their 0.2 g doses were significantly better than the placebo, with 0.1 g amylobarbitone sodium also reaching a significant level. Although the barbiturates became significantly different from the placebo at different times, at no time was there a significant difference between the 0.2 g doses of any two barbiturates.

Comparison of scores for the second 4 hr of the night would show if any of the barbiturates had a more sustained effect. Butobarbitone 0.2 g and amylobarbitone sodium 0.2 g maintain their significant effect; quinalbarbitone sodium 0.2 g just fails to reach the level of significance when compared with both placebo nights, but it is not significantly different from the other two barbiturates tested.

The total 8-hr movement score (Tables 1 and 2) is, as expected, reduced by the barbiturates; the difference between the 0.2 g doses and the placebo easily reaches the 1% level of significance. Throughout the night the 0.1 g doses give results intermediate between 0.2 g and placebo, rarely achieving a statistically significant difference from placebo.

Nurses' record. Although the nurses did not know which, if any, barbiturate was in the capsules, they assessed the patients' length of sleep such that the mean was 7.09 hr for butobarbitone 0.2 g and 7.06 hr for both amylobarbitone and quinalbarbitone sodium 0.2 g. The means for the two placebo nights were 5.66 and 5.88 hr, significantly different when subjected to the same statistical tests as the movement score. These results (Table 3) parallel the results for the motility score in that patients who are asleep move less. It may be noted that levels of statistical significance are less readily reached by comparison of sleep estimations,

the probability of the difference between placebo and 0.2 g barbiturate being a chance one is between 5% and 1%, whereas the movement score shows a probability of less than 1%.

Sleep was broken less frequently when the patient received a barbiturate than when he took the placebo, the larger dose being more effective (see Table 3). The difference was statistically significant ($P<0.05$) between placebo and 0.2 g of butobarbitone and quinalbarbitone sodium.

The time taken to get to sleep was, as expected, less with barbiturates than without. However, the individual variation and scatter was so wide in this group of disturbed patients (the times ranged from 5 min to 3¾ hr) that in the analysis of variance the difference was not significant. Table 4 shows the proportion of

TABLE 4

PERCENTAGE OF PATIENTS ASLEEP 15, 30, 45 AND 60 MIN AFTER 0·2 G BARBITURATE COMPARED WITH PLACEBO

(*=$P<0·02$ using arc sin transformation)

	Butobarb.	Quinalbarb. sod.	Amylobarb. sod.	Placebo
After 15 min	25%	31%	25%	22%
After 30 min	44%	68%*	50%	32%
After 45 min	94%*	81%	94%*	56%
After 1 hr	94%*	94%*	94%*	72%

subjects asleep at the end of each quarter-hour for the first hour; the arc sin transformation of the square roots of the proportions (Davies, 1954) was used to assess the significance of differences. Compared with the placebo, this proportion becomes significantly greater for quinalbarbitone sodium after 30 min (C.R.=2.34, $P<0.02$) and amylobarbitone sodium and butobarbitone after 45 min (C.R=2.45, $P<0.02$).

Patients' record. The patients thought their sleep sounder when the capsules contained a barbiturate. Rating "sound" as 1, "fair" as 2, "poor" as 3, and "very poor" as 4, the results were again found to be significant when subjected to analysis of variance. The difference from placebo was significant with quinalbarbitone sodium 0.2 g ($P<0.01$) and amylobarbitone sodium 0.2 g ($P<0.05$), but with butobarbitone it just failed to reach the 5% level.

The patients who said that they had a "hang-over" usually described it as a feeling of heaviness, tiredness, slight giddiness or a headache. Inspection of Table 3 shows that this "hang-over" was reported 2 to 4 times with each barbiturate in both doses and also with placebo, and it is apparent that its incidence was not significantly related to barbiturates in this trial.

The pattern of sleep. The motility score per hour, illustrated as a histogram in Fig. 2, indicates the distribution of restlessness throughout the night. Without the barbiturate (broken line in histogram) this group of patients with insomnia moved most in the first hour whilst seeking sleep; in the next few hours they moved considerably less with variation from hour to hour and towards 6 a.m. movement increased again. With the barbiturates the pattern is similar, although quantitatively different. It is apparent that butobarbitone 0.2 g is effective throughout the 8 hr and, notably, the first 1 hr, although what little effect the 0.1 g

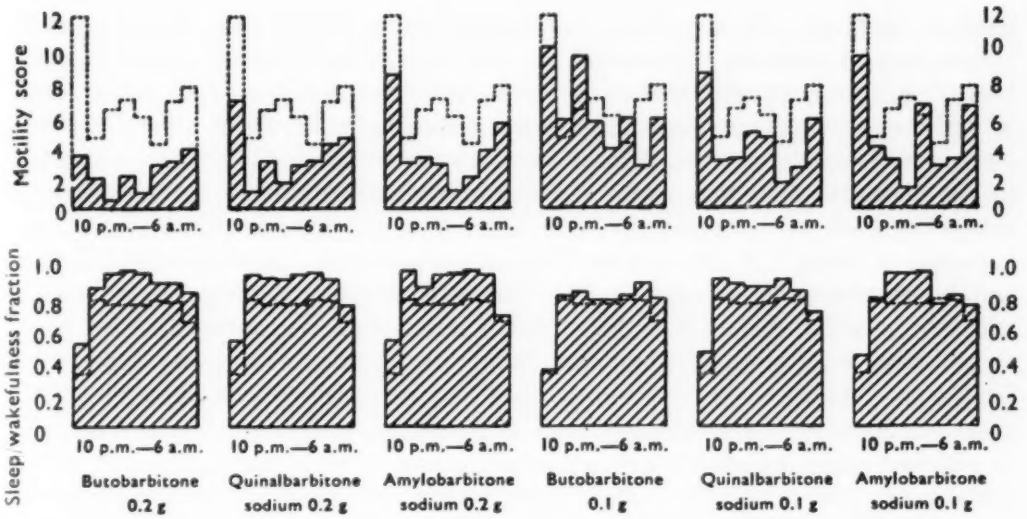

Fig. 2. The alteration of movement and recorded sleep produced by the three barbiturates (hatched) in 0·2 g and 0·1 g doses as compared with placebo (broken line). Upper histograms; mean motility score for each hour between 10 p.m. and 6 a.m. Lower histograms: mean sleep/wakefulness fraction for each hour (a score of 1·0 indicates that all the patients slept throughout that hour).

dose has is in the latter part of the night. The action of quinalbarbitone and amylobarbitone sodium is soon discerned and again persists until morning. The record of the patients' sleep made by the nurses provides a corresponding pattern if it is illustrated as a sleep/wakefulness fraction per hour, such that a score of 1.0 is attained if all 16 patients slept the whole hour. The barbiturates increased the amount of sleeping at the same time as they decreased the movement, although the alteration of motility is greater.

DISCUSSION

The measurement of movement during the night is a practicable method of obtaining an indication of the hypnotic effect of drugs and correlates with the evaluation of sleep by the nurses and patients. Although a more direct method of measuring sleep would be welcome, this at least avoids disturbing the patients' rest, gives a reliable score and does not ask for too much co-operation from disturbed psychiatric patients, in comparison with other methods discussed in previous papers (Cox & Marley, 1959; Hinton & Marley, 1959). As expected, the trial confirmed that 0.2 g of these three barbiturates were efficient hypnotic agents, inducing sleep earlier, increasing the amount of sleep obtained between 10 p.m. and 6 a.m., reducing restlessness and the interruptions of sleep; whilst the patient feels that he has slept more soundly without any increase in unwelcome bodily sensations on waking.

The main purpose of the trial was to compare the action of these three barbiturates as there are surprisingly few controlled studies on the production of sleep by barbiturates in human subjects. Animal experimentation inducing anaesthesia is not a satisfactory substitute, nor is the estimation of blood or plasma barbiturate

level, as there may be considerable differences between the comparative rates of metabolism of different barbiturates in man and animals (Brodie, Burns, Mark, Lief, Bernstein & Papper, 1953); moreover the relationship between blood levels of barbiturates and anaesthesia is not very exact (Raventós, 1954). Tatum (1939), when discussing the now traditional method of classifying barbiturates according to the duration of action, wrote, "Experimentalists are forced to depend upon symptoms such as incoordination and various stages of anaesthesia rather than hypnosis or sleep." In the *Extra Pharmacopœia* (1958), amylobarbitone sodium and butobarbitone are placed in the intermediate group, acting within 30 min and having a duration of 5 to 6 hr, whilst quinalbarbitone sodium is said to be short-acting with its action apparent in 15 to 20 min and lasting 2 to 4 hr. Goodman & Gilman (1955) write that the short-acting barbiturates produce less than 3 hr hypnosis. Differing emphasis is placed upon the reputedly slower onset of action of the intermediate-acting derivatives, but Tatum (1939) had said of the barbiturates, "It so happens that, with the exception of barbital and phenobarbital, practically all of them have a short induction period." In the last decade doubts have been expressed concerning the claimed periods of action of the various barbiturates. In 1951 Goodnow, Beecher, Brazier, Mosteller & Tagiuri, in testing for impaired physiological performance in man after 0.1 g of pentobarbitone sodium, found this trend still present after 14 hr. In 1956 Lasagna, noting the absence of clinical data bearing on the accepted classification of the barbiturates, showed clearly that in a group of chronically ill patients quinalbarbitone (secobarbital) sodium and pentobarbitone sodium had a similar onset and duration of effect.

The results of this trial show that there is little clinical difference between the effects of the one short-acting and the two intermediate-acting barbiturates. With 0.2 g of each barbiturate a decrease in restlessness becomes apparent in the first hour, notably with butobarbitone, one of the intermediate group. All three drugs have an effect throughout the night; at no time is there a significant difference between the 0.2 g doses, although sometimes one and sometimes another barbiturate reaches a different level of statistical significance. This also applies to the length of sleep, the number of times it was broken and the patients' estimate of its soundness. It might be said that the 0.2 g dose was so large as to obscure the finer differences between the three barbiturates, but 0.1 g was in most cases insufficient to produce a significant difference from placebo in these disturbed patients. Amylobarbitone and quinalbarbitone sodium 0.1 g produced a qualitatively similar sleep and movement pattern to the 0.2 g dose, and all that can be said of butobarbitone 0.1 g is that, in marked contrast to 0.2 g, it was not as ineffective in the latter part of the night as in the first part. In many of the assessments 0.2 g of butobarbitone had the greatest effect, but it is to be remembered that it contains almost 10% more of the barbiturate base than 0.2 g of the sodium salts of amylobarbitone and quinalbarbitone. It would be justifiable to say that variation in the severity of disturbance in psychiatric patients hides the differences between the drugs. This factor, randomized by the design of the trial, played a part in the failure to reach a statistically significant difference in the times taken to get to sleep. Stewart (1956) had a similar finding in a clinical trial using buto

barbitone and amylobarbitone sodium in ward patients. The variation between these mentally ill individuals was great, always more than that between the drugs, and having 2 patients who moved little in the second group of 8 patients produced some variance between the two groups, hence the significant difference between squares in Table 1. Nevertheless, in Lasagna's (1956) trial with chronically ill patients, where the individual's insomnia was presumably more constant, similar conclusions were reached.

The possibility was considered that, once a barbiturate had induced sleep, the patient stayed asleep and the drug with a short action could not be distinguished from one with intermediate action. If the hypnotic action were short, a patient taking quinalbarbitone sodium 0.2 g and waking in the first part of the night would be more restless in the second half of the night than the mean of all the patients receiving this drug. In fact, 6 such patients awoke between midnight and 2.30 a.m., and their mean motility score for the first half of the night was 15 (mean for all those taking quinalbarbitone sodium was 12.5). The mean for these 6 in the second four hours of the night was 14.2, compared with 14.4 for the whole group (placebo group 24.7). This trend indicates that quinalbarbitone sodium was still effectively inducing sleep again once the patient had woken.

It is clear that the accepted grouping of the short-acting and intermediate-acting barbiturates is, as yet, not borne out by controlled studies of their hypnotic action in man.

I wish to thank Dr. D. L. Davies, whose help and encouragement made this investigation possible; also Dr. A. E. Maxwell for his advice on statistics; Mr. G. H. Cox for his technical assistance, including the construction of the recording apparatus; Sister Kvass and the nursing staff of Ward 4 for their careful recording; and May & Baker for supplying the barbiturates.

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EFFECT OF ANTIBIOTICS ON THE 5-HYDROXYTRYPTAMINE CONTENT OF THE SMALL INTESTINE AND OTHER ORGANS IN RATS AND MICE

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(*Received October* 13, 1960)

Rats and mice were given antibiotics orally and by subcutaneous injection and the effects on tissue levels of 5-hydroxytryptamine and intestinal bacteria were studied. In mice it was found that antibiotics which caused a large reduction in the bacterial flora of the intestine when given orally also caused a significant increase in intestinal 5-hydroxytryptamine. In rats, neomycin caused a reduction in the urinary excretion of 5-hydroxyindoleacetic acid. In both rats and mice, many antibiotics caused a significant reduction in the weight of the spleen.

Stacey & Sullivan (1957) reported that the intestinal content of 5-hydroxytryptamine in mice was raised after oral administration of a combination of streptomycin and chlortetracycline. They found that rectal swabs from treated mice taken on the day of death were sterile, and suggested that sterilization of the gut might have increased the amount of tryptophan available for 5-hydroxytryptamine synthesis by preventing the bacterial metabolism of dietary tryptophan. The present paper reports further experiments undertaken to amplify these results.

METHODS

Groups of adult male mice weighing 20 to 30 g and of rats weighing 130 to 180 g were matched according to weight and age, and were litter-mates where possible. Control and treated groups received a mixed, balanced diet in cubes, and water *ad lib.*

Antibiotics were given twice daily either orally by stomach tube or by subcutaneous injection. The doses used, roughly five times the adult human therapeutic dose in mg/kg, were as follows:

(1) Neomycin, 215 mg/kg, for rats and mice. Solution for rats 50 mg/ml. and for mice 25 mg/ml.
(2) Streptomycin, 70 mg/kg in a solution of 10 mg/ml. for all animals.
(3) Chlortetracycline and oxytetracycline, 70 mg/kg, in a solution of 10 mg/ml. for all animals.
(4) Chloramphenicol, 105 mg/kg, in a solution of 15 mg/ml. for all animals.

When combinations of antibiotics were used, the same doses of the individual drugs were given. Solutions of drugs were made up, when necessary, in normal saline. Controls were given the same volume of normal saline.

Animals were killed by stunning and bleeding. The whole of the spleen and brain was homogenized and aliquots taken for assay of 5-hydroxytryptamine in both rats and mice. The small intestine of mice, from the pyloric sphincter to the ileo-caecal junction, was similarly treated. The mucous membrane of the small intestine of rats was scraped off with a scalpel

and homogenized. This contains all the 5-hydroxytryptamine (Feldberg & Toh, 1953) and is easier to homogenize than the whole small intestine of the rat.

Blood was obtained from mice for estimation of platelet 5-hydroxytryptamine as previously described (Sullivan, 1960). Platelet-rich plasma was prepared as described by Hardisty & Stacey (1955); the platelets were counted in whole blood and platelet-rich plasma by the method of Baar (1948).

The methods of Udenfriend, Weissbach & Clark (1955) and Weissbach, Waalkes & Udenfriend (1958) were used for the fluorimetric assay of 5-hydroxytryptamine in aliquots of intestine and spleen homogenates. In platelet-rich plasma, 5-hydroxytryptamine was assayed by the method of Udenfriend *et al.* (1955) and for brain 5-hydroxytryptamine the fluorimetric method of Bogdanski, Pletscher, Brodie & Udenfriend (1956) was used. Interference by tryptamine and tryptophan was excluded by measuring the fluorescence in 3 N hydrochloric acid, in which these substances do not fluoresce (Udenfriend, Bogdanski & Weissbach, 1955).

The 5-hydroxytryptamine content of blood platelets is expressed as μg/ml. blood, the figures being derived from the results of assays of platelet-rich plasma as described by Hardisty & Stacey (1955). Results of 5-hydroxytryptamine in other tissues are expressed in μg/kg body-weight rather than in μg/g for reasons previously discussed (Sullivan, 1960). Results expressed in μg/g are similar, but in some instances less reliable. There was some variation between the results obtained for control groups because of differences in body-weight between them.

Urinary excretion of 5-hydroxyindoleacetic acid was estimated on pooled rat urine by the method of Marfarlane, Dalgliesh, Dutton, Lennox, Nyhus & Smith (1956), after collection of urine from rats in metabolism cages for 16 to 18 hr overnight. Urinary creatinine was estimated by the method of Folin (1914).

Details of techniques used for experiments on the recovery of injected 5-hydroxytryptamine as 5-hydroxyindoleacetic acid, for observation of the rate of loss of 5-hydroxytryptamine from incubated pieces of jejunum and for bacteriological studies, have been described previously (Sullivan, 1960).

Throughout the paper means are shown with their standard errors.

RESULTS

Experiments with mice

Results of 5-hydroxytryptamine assays. Table 1 shows the results obtained when streptomycin and chlortetracycline were given orally and by subcutaneous injection to mice for 9 to 11 days. After oral administration, there was a significant rise of intestinal 5-hydroxytryptamine in treated mice, but there was no difference between treated and control groups after subcutaneous injection, although a smaller number of animals were used. The spleen content of 5-hydroxytryptamine was unaffected in both experiments.

TABLE 1

EFFECT OF STREPTOMYCIN AND CHLORTETRACYCLINE GIVEN FOR 9 TO 11 DAYS ON THE 5-HYDROXYTRYPTAMINE CONTENT OF THE TISSUES OF MICE

Mean results are expressed as μg/kg 5-hydroxytryptamine $\pm$ standard error. n.s. = difference not significant ($P > 0.05$)

Administration of drugs	Tissue examined	No. of mice		5-Hydroxytryptamine content		
		Control	Treated	Control	Treated	Significance
Orally	Small intestine	17	17	131·5 $\pm$ 9·1	172·0 $\pm$ 11·8	$P < 0.02$
	Spleen	10	11	69·0 $\pm$ 6·4	69·0 $\pm$ 5·0	n.s.
Subcutaneous injection	Small intestine	7	9	154·0 $\pm$ 19·7	204·0 $\pm$ 25·2	n.s.
	Spleen	6	7	93·0 $\pm$ 10·9	86·7 $\pm$ 20·0	n.s.

Neomycin was given orally to mice, but not parenterally in view of its severe systemic toxicity (Waisbren, 1956). Table 2 shows the results of two experiments of different duration. In both there was a significant rise of intestinal 5-hydroxytryptamine, while the spleen content was significantly raised in the shorter experiment lasting 9 to 11 days. Brain 5-hydroxytryptamine was unaffected in both experiments. There was a significant rise of platelet 5-hydroxytryptamine after 9 to 23 days of treatment. These assays were spread over a longer period of time owing to the technical difficulties of doing more than a limited number of assays at any one time.

TABLE 2

EFFECT OF NEOMYCIN GIVEN ORALLY ON THE 5-HYDROXYTRYPTAMINE CONTENT OF THE TISSUES OF MICE

Mean values of 5-hydroxytryptamine are expressed as μg/kg $\pm$ standard error, except for platelets, where the results are expressed as μg/ml. blood. n.s.=difference not significant ($P>0.05$)

Duration of treatment (days)	Tissue examined	No. of mice		5-Hydroxytryptamine content		
		Control	Treated	Control	Treated	Significance
9 to 11	Small intestine	10	9	127·5±15·6	209·0±28·9	$P<0.05$
	Spleen	10	9	58·5±7·2	98·0±7·2	$P<0.01$
	Brain	8	8	15·3±0·84	16·8±1·02	n.s.
20 to 21	Small intestine	9	10	196·0±11·3	252·0±12·9	$P<0.01$
	Spleen	9	9	95·5±14·0	144·5±24·2	n.s.
	Brain	8	10	16·5±0·54	15·4±0·57	n.s.
9 to 23	Blood platelets	13	16	3·23±0·17	4·42±0·47	$P<0.05$

Chlortetracycline was given orally for 10 to 20 days. It did not affect the 5-hydroxytryptamine content of the spleen or brain but caused a significant rise of intestinal 5-hydroxytryptamine. The mean values of 5-hydroxytryptamine were 163.0±7.72 μg/kg for 31 control mice and 192.0±9.96 μg/kg ($P<0.05$) for 33 treated mice. Tissue levels of 5-hydroxytryptamine in the small intestine and brain were not significantly affected by subcutaneous injection for 10 to 20 days.

The following antibiotics, singly or combined, were given orally for 10 to 20 days, and all but the last two were also given by subcutaneous injection: streptomycin and chloramphenicol; streptomycin and oxytetracycline; oxytetracycline; streptomycin; chloramphenicol. The 5-hydroxytryptamine content of the small intestine and spleen was not significantly altered by any of these drugs by either method of administration.

Effect upon the weight of the spleen. During the course of these experiments it was observed that the weight of the spleen in mice treated with certain antibiotics, orally and parenterally, was less than in controls. This is shown in Table 3. The weight of the spleen in treated mice was reduced in all experiments except those in which neomycin and streptomycin were given. The weight reduction was significant in all cases except in the experiment in which streptomycin and oxytetracycline were given together orally.

The spleens of mice treated with the combination of streptomycin and chlortetracycline orally were compared histologically with the spleens of control mice.

TABLE 3

EFFECTS OF ANTIBIOTICS ON THE WEIGHT OF THE SPLEEN IN MICE

Mean weights are given in g±standard error. n.s.=difference not significant ($P>0.05$)

Antibiotics	Method of administration	No. of mice		Weight of spleen		
		Control	Treated	Control	Treated	Significance
Streptomycin and chlortetracycline	Oral	10	11	0·36±0·03	0·19±0·01	$P<0·001$
	Injection	6	7	0·23±0·03	0·13±0·02	$P\simeq0·02$
Streptomycin and chloramphenicol	Oral	12	12	0·20±0·02	0·10±0·01	$P<0·001$
	Injection	8	10	0·31±0·05	0·14±0·02	$P<0·01$
Streptomycin and oxytetracycline	Oral	6	8	0·35±0·02	0·25±0·06	n.s.
	Injection	6	6	0·23±0·03	0·13±0·02	$P<0·02$
Chlortetracycline	Oral	15	14	0·25±0·03	0·16±0·01	$P<0·01$
Chloramphenicol	Oral	9	9	0·45±0·08	0·27±0·03	$P<0·05$
Neomycin	Oral	19	18	0·32±0·03	0·34±0·04	n.s.
Streptomycin	Oral	10	11	0·36±0·03	0·39±0·06	n.s.

TABLE 4

COMPARISON OF THE EFFECTS PRODUCED BY ANTIBIOTICS GIVEN ORALLY ON THE BACTERIAL FLORA, TISSUE 5-HYDROXYTRYPTAMINE AND SPLEEN WEIGHT IN MICE

† denotes $P<0.05$. The figures for statistically significant results have been given in the text or in Tables 1–3

Bacterial flora	Antibiotics	% change in total content of 5-hydroxytryptamine		% change in weight of spleen
		Intestine	Spleen	
Sterile	Neomycin (2 experiments)	+45·5†	+58·5†	+5
Sterile or partly suppressed	Streptomycin and chlortetracycline	+31†	0	−67†
	Streptomycin and chloramphenicol	+18	−12	−47·5†
Partly suppressed and altered	Chlortetracycline	+18†	+13	−36†
	Streptomycin	+18·5	+22	+9·5
	Chloramphenicol	+4·5	−2·5	−40†
	Streptomycin and oxytetracycline	+1	−11·5	−28
Altered only	Oxytetracycline	+5	—	—

No difference was observed between the two groups, the architecture and the proportion of lymphoid tissue being the same.

Bacteriological studies. Studies of bacteria grown from rectal swabs showed that, compared with controls, the flora grown from treated animals could be classified into four main groups:

(1) Complete sterility; no growth obtained on aerobic or anaerobic plates.

(2) Complete sterility in some treated animals and a partial suppression of bacterial growth in others.

(3) The intestine was not made sterile in any of the animals. The bacterial flora was partially suppressed in some of the treated mice, while in others the flora was altered qualitatively, but there was no obvious reduction in the number of colonies grown.

(4) The bacterial flora altered qualitatively, but no obvious reduction in the number of colonies grown.

Table 4 shows the bacteriological results obtained in mice treated with the various antibiotics orally, and relates these findings to the percentage changes in the 5-hydroxytryptamine content of intestine and spleen, and to the percentage changes in spleen weight. It will be seen that, in general, the more potent the antibacterial action of the antibiotic or combination of antibiotics used, the greater was the effect on tissue levels of 5-hydroxytryptamine. On the other hand, the effect on intestinal bacteria was apparently unrelated to the effect produced on the weight of the spleen.

In Table 5, similar results are shown for those experiments in which antibiotics were administered parenterally. It will be seen that the antibacterial effect was much

TABLE 5

COMPARISON OF THE EFFECTS PRODUCED BY ANTIBIOTICS GIVEN BY SUBCUTANEOUS INJECTION ON THE BACTERIAL FLORA, TISSUE 5-HYDROXYTRYPTAMINE AND SPLEEN WEIGHT IN MICE

† denotes $P<0.05$. The figures for statistically significant results have been given in the text or in Tables 1–3

Bacterial flora	Antibiotics	% change in total content of 5-hydroxytryptamine: Intestine	% change in total content of 5-hydroxytryptamine: Spleen	% change in weight of spleen
Partly suppressed and altered	Streptomycin and chlortetracycline	+32·5	−35	−43†
	Streptomycin and chloramphenicol	−7·5	−39·5	−54·5†
Altered only	Chlortetracycline	0	—	—
	Streptomycin and oxytetracycline	−14	−39	−45·5†
	Oxytetracycline	+1	—	—

less than when the antibiotics were given orally, and so also was the effect on tissue levels of 5-hydroxytryptamine, but again, neither of these factors appeared to be related to the effect upon the weight of the spleen in treated mice.

Condition of the mice. In general, the condition of control and treated groups of mice remained satisfactory. In nearly all experiments, the control groups gained up to 10% of their body-weight. The effect of antibiotics on the body-weight of treated mice varied, some treated groups gaining up to 12% during the experiment, others losing a similar amount of weight.

There was no relationship between changes in the body-weight of treated mice and the effect of antibiotics on spleen weight. For instance, mice treated orally with chloramphenicol gained 11.5% of their body-weight during the experiment, their mean body-weight at the end being greater than that of the control group which gained 6% of their body-weight during the experiment. But the mean spleen weight of the chloramphenicol-treated group was significantly lower than that of the control mice.

There was no significant difference in the weight of the small intestine between control and treated groups in any of the experiments.

Experiments with rats

Results of 5-hydroxytryptamine assays. Rats were treated with a combination of streptomycin and chlortetracycline orally for 8 to 9 days and with neomycin orally for 21 to 25 days. No significant effect was obtained on the 5-hydroxytryptamine content of the small intestine in either experiment, although the intestinal 5-hydroxytryptamine content of 7 rats given streptomycin and chlortetracycline showed a mean rise of 36% compared with 5 controls, while that of 6 neomycin-treated animals rose 18% compared with 6 controls.

Effect upon the weight of the spleen. The mean spleen weight of 6 rats treated with streptomycin and chlortetracycline was 0.39 ± 0.03 g and of 5 controls 0.53 ± 0.08 g. Although the spleen weight was lower in treated rats than in controls, the difference was not significant.

Bacteriological studies. In both of these experiments, the antibiotics used caused either sterility or partial suppression of the bacterial flora. Streptomycin with chlortetracycline caused a similar degree of antibacterial action in mice, but the antibacterial effect of neomycin was less potent than in mice, in which it caused sterility in all animals.

TABLE 6

URINARY EXCRETION OF 5-HYDROXYINDOLEACETIC ACID BY 10 CONTROLS AND 10 RATS DURING AND AFTER TREATMENT WITH NEOMYCIN GIVEN ORALLY

Standard errors are given with mean results

Duration of treatment (days)	5-Hydroxyindoleacetic acid μg/kg/24 hr		Creatinine mg/kg/24 hr		Ratio of 5-hydroxyindoleacetic acid/creatinine	
	Control	Treated	Control	Treated	Control	Treated
7	146·0	78·5	28·4	25·1	5·2	3·1
15	228·5	94·5	29·8	24·7	7·7	3·8
17	155·0	84·5	28·2	25·4	5·5	3·3
23	110·0	62·0	32·0	25·0	3·5	2·5
Means	160·0 ±24·6	80·0 ±6·9				
	$P < 0.05$					
Days after treatment ended 14	134·0	174·0	30·0	33·2	4·5	5·3
Days after treatment ended 21	156·0	153·0	29·0	27·6	5·4	5·6
Means	145·0 ±7·7	164·0 ±7·4				

Urinary excretion of 5-hydroxyindoleacetic acid. No difference was found between treated and controls when rats were given streptomycin with chlortetracycline, but neomycin treatment caused a significant reduction of 5-hydroxyindoleacetic acid excretion as shown in Table 6. The level of excretion was similar to that of the control group, 14 and 21 days after neomycin administration had been stopped, however.

Additional experiments in antibiotic-treated animals

Incubation of jejunum. Pieces of jejunum from neomycin-treated and control mice were incubated *in vitro* in Krebs Ringer phosphate solution for up to 2 hr.

At intervals of 30 min the 5-hydroxytryptamine content was assayed to study the rate of release from the jejunum. No difference was found between the groups, and both had lost about 50% of the tissue content of 5-hydroxytryptamine by the end of the 2-hr period.

Recovery of injected 5-hydroxytryptamine. Antibiotic-treated and control groups of rats were given 6 mg/kg 5-hydroxytryptamine by subcutaneous injection and the urinary recovery of the amine as 5-hydroxyindoleacetic acid was studied. There was no significant difference between treated and control groups. The recovery from 7 rats treated with streptomycin and chlortetracycline was 34% and from 5 controls 31%. From 7 neomycin-treated rats recovery was 26.5% and from 6 controls 29.5%.

DISCUSSION

With the exception of neomycin, antibiotics only affected the 5-hydroxytryptamine content of the intestine in mice. The absence of any effect on 5-hydroxytryptamine in other tissues indicates that this was a local effect only, due possibly to increased storage of 5-hydroxytryptamine. Neomycin caused a rise of 5-hydroxytryptamine in the spleen and platelets, in addition to the intestine. This might have been due to the fact that the capacity of the intestine for storing 5-hydroxytryptamine was exceeded in neomycin-treated mice, resulting in the release of excess of the amine into the circulation. The results of incubation of jejunum *in vitro,* from which about the same amount of 5-hydroxytryptamine was lost in neomycin-treated and control mice, do not support this explanation, but these findings may not apply *in vivo.* Another possibility is that neomycin caused an increase in 5-hydroxytryptamine synthesis in the tissues. Since very little neomycin is absorbed from the lumen of the intestine, this is unlikely to have occurred.

The results suggest that the effect on intestinal 5-hydroxytryptamine was mediated by the effects the antibiotics produced on intestinal bacteria. After oral administration, the more effective the antibacterial action, the greater was the effect on tissue levels of 5-hydroxytryptamine. When these antibiotics are given by injection, only a small proportion reaches the intestine via the biliary tract (for references see Sollmann, 1957), and it was found that the effect of antibiotics on intestinal bacteria was much less potent after subcutaneous injection than when they were given orally. The antibiotics also had a greater effect on tissue levels of 5-hydroxytryptamine after oral administration than after injection

If this explanation of the mechanism of action of these antibiotics is correct, it is analogous to the effects of antibiotics in promoting growth in various animal species, which is believed to be due to alterations in the bacterial flora of the intestine (Stokstad, 1954). Results obtained from growth experiments suggest that inhibition of bacteria might release essential nutrients for the use of the host, which would otherwise be metabolized for use by bacteria. This mechanism might also account for the rise of tissue 5-hydroxytryptamine in antibiotic-treated mice. It is suggestive that Linkswiler, Baumann & Snell (1951) found that inhibition of intestinal bacteria in rats by chlortetracycline prevented destruction of dietary pyridoxal. A similar, tryptophan-sparing, action was found by Jones & Combs (1951) in chlortetracycline-fed chicks.

There is evidence that some antibiotics are capable of producing pharmacological effects (Nakatsuka & Matsumoto, 1952 ; Rokos, Burger & Procházka, 1958 ; Sobek, 1959), and the possibility that the effects on tissue 5-hydroxytryptamine were due to the direct pharmacological actions of antibiotics cannot be excluded. This seems unlikely, however, especially since neomycin, which produced the greatest effect, is absorbed only in negligible amounts from the intestine.

The effect of antibiotics upon the spleen weight in mice occurred after both oral administration and subcutaneous injection. It was unrelated both to the effect on tissue levels of 5-hydroxytryptamine and to the antibacterial effects of the drugs. The latter makes it unlikely that the effect was mediated by alterations in the intestinal bacterial flora, and since it has been shown that the size of the spleen is unchanged in germ-free animals compared with controls (Stokstad, 1954), it is unlikely that the phenomenon was due to the prevention or suppression of systemic infections. The most likely explanation is that the reduction in spleen weight was due to direct action by the antibiotics used, and this is supported by the fact that those antibiotics which are not absorbed after oral administration, namely, neomycin and streptomycin, did not reduce the spleen weight.

In rats, the effects of antibiotics on intestinal 5-hydroxytryptamine were similar to those in mice, but the results were not significant at the 5% level. This may have been because the numbers of rats used were too small. Alternatively, if alteration of intestinal 5-hydroxytryptamine is dependent upon changes in the intestinal flora involving specific strains of bacteria, this species variation may be accounted for by differences in the occurrence of these strains in rats and mice.

Anderson, Ziegler & Doeden (1958) reported that when neomycin was given to monkeys in sufficient doses to produce sterilization of the intestine, urinary excretion of 5-hydroxyindoleacetic acid was unaffected. In the experiments described above, neomycin caused a significant reduction in the urinary excretion of 5-hydroxyindoleacetic acid in rats, although streptomycin with chlortetracycline did not affect excretion of this metabolite. Since no significant difference was found in the percentage of injected 5-hydroxytryptamine recovered as 5-hydroxyindoleacetic acid between neomycin-treated and control rats, it is unlikely that neomycin caused any alteration in metabolism.

I wish to thank Professor R. Hare for facilities and advice on bacteriological studies, Dr. H. Spencer for assistance with histological studies and Professor R. S. Stacey for helpful advice and criticism of this paper.

Approved as part of a thesis for the degree of Doctor of Philosophy of the University of London.

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FACTORS INFLUENCING THE HYPERTENSIVE EFFECT OF ESERINE IN THE RAT

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(*Received October* 31, 1960)

Several factors influencing the hypertensive effect of eserine in the rat were investigated. Pretreatment with reserpine regularly depressed or abolished the hypertensive response to eserine. The slow intravenous infusion of either noradrenaline, dihydroxyphenylalanine or 5-hydroxytryptamine only occasionally restored the hypertensive effect of eserine in reserpine-treated rats. Bretylium and choline 2,6-xylyl ether bromide significantly depressed or even abolished the hypertensive effect of eserine. The effect of bretylium was stronger than that of choline 2,6-xylyl ether bromide. Cocaine was found to antagonize the action of bretylium on the response to eserine. In doses which significantly depressed the action of eserine bretylium did not inhibit the hypertension due to excitation of medullary centres induced by clamping the common carotid arteries. Lowering of body temperature abolished the hypertensive effect of eserine. Pretreatment with isopropylisoniazid did not antagonize the inhibitory action of reserpine on the hypertensive response to eserine. It is concluded that the present experiments indicate that the hypertensive effect of eserine in the rat is due to central activation of adrenergic nervous elements. Liberation of noradrenaline (and adrenaline) from the adrenals and from the blood vessels by eserine is an insignificant factor in producing the hypertensive response to eserine.

Eserine raises the blood pressure of the rat anaesthetized with urethane (Varagic, 1955; Dirnhuber & Cullumbine, 1955; Hornykiewicz & Kobinger, 1956) as well as of the conscious rat (Medakovic & Varagic, 1957). This hypertensive effect was attributed to the discharge of impulses from centres in the central nervous system. On the other hand, as long ago as 1921, Stewart and Rogoff observed that in cats the output of adrenaline from the adrenals was increased 10 to 15 times after eserine. In the rat, however, liberation of adrenaline (and noradrenaline) from the adrenals does not seem to play a significant role in the blood pressure rise after eserine because the hypertensive effect of eserine was very much the same in adrenalectomized rats as in the controls (Medakovic & Varagic, 1957).

There is evidence that noradrenaline is present in the walls of the blood vessels (Schmiterlöw, 1948; Burn & Rand, 1958a). This noradrenaline could be liberated by treatment of the animal with reserpine and again restored by an intravenous infusion of noradrenaline (Burn & Rand, 1958a). In reserpine-treated cats some sympathomimetic amines lose their hypertensive activity (Carlsson, Rosengren, Bertler & Nilsson, 1957) which could be restored by intravenous infusion of noradrenaline (Burn & Rand, 1958b). The hypertensive effect of eserine in the reserpine-treated rat is also depressed or abolished (Hornykiewicz & Kobinger, 1956),

but it is not known whether intravenous infusion of noradrenaline or its precursor might restore that effect. Experiments have now been done in order to determine to what extent a peripheral liberation of noradrenaline might contribute to the hypertensive effect of eserine in the rat. By using bretylium, a new type of adrenergic blocking agent, and also by lowering the temperature of the animal, it was possible to obtain some new data which might explain the hypertensive response to eserine.

METHODS

Rats of both sexes (150 to 380 g) were used and anaesthetized with 0.7 ml./100 g body weight of 25% urethane solution injected subcutaneously. To record the blood pressure a cannula was inserted into the carotid artery and connected with a capillary mercury manometer (Condon, 1951). A small polythene cannula, 0.5 mm in diameter, was inserted into the jugular vein and was used for injecting drugs. Before the experiment was started 1 to 1.5 mg/100 g body weight of heparin was injected. All doses of drugs were injected in 0.1 ml. and washed in with the same volume of saline.

Noradrenaline and l-dihydroxyphenylalanine were infused from a microburette through a small polythene cannula in the jugular vein. The rate of infusion ranged from 100 to 1,000 ng/min for noradrenaline, and was 0.25 mg/min for dihydroxyphenylalanine. Reserpine was injected intraperitoneally in a dose of 1 to 2 mg/kg on 2 successive days and on the third day the animal was used for experiment.

Cooling was done by placing pieces of ice over the body of the anaesthetized animal.

The following substances were used: noradrenaline bitartrate, l-dihydroxyphenylalanine, eserine salicylate, cocaine hydrochloride, bretylium tosylate, choline 2,6-xylyl ether bromide, isopropylisoniazid and reserpine ("Serpasil," Ciba).

RESULTS

The effect of noradrenaline on the response to eserine. It was found that previous intravenous infusion of noradrenaline in doses from 1 to 10 μg for 10 min does not significantly alter the blood pressure response to eserine. Only in 3 out of 9 experiments was a slight potentiation of the response to eserine observed. In 3 other experiments the effect of eserine was depressed after infusion of noradrenaline, whereas in the remaining 3 no change was observed.

The effect of reserpine and noradrenaline on the pressor response to eserine. Pretreatment of rats by reserpine in doses from 1 to 2 mg/kg intraperitoneally significantly depressed or abolished the hypertensive response to eserine. These findings confirm those of Hornykiewicz and Kobinger (1956). In reserpine-treated animals the noradrenaline stores in the blood vessels are presumably low. If eserine acted by liberating noradrenaline from the stores it should be possible to restore the response to eserine by slow intravenous infusion of noradrenaline. It was found in the present work that only in 2 out of 11 experiments did infusion of noradrenaline restore the hypertensive effect of eserine, whereas in 9 experiments no change was observed. One of the 2 experiments is shown in Fig. 1, where the usual response to 41.7 μg/kg eserine in the reserpine-treated animal is shown in (*a*). Between the arrows 5 μg noradrenaline was infused slowly for 10 min into the jugular vein. After infusion had stopped and after the rise of blood pressure which it caused had subsided, the intravenous injection of eserine now caused a significant rise in blood pressure, as shown in (*b*). Two hours after infusion of noradrenaline the response to eserine again disappeared, as shown in (*c*).

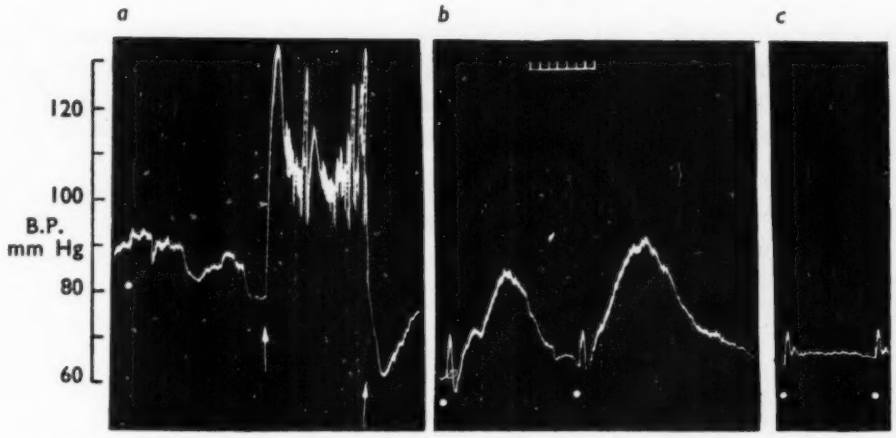

Fig. 1. The effect of eserine and slow intravenous infusion of noradrenaline on the blood pressure in a reserpine-treated rat (240 g). At white dots: 10 μg eserine intravenously. Between the arrows: slow intravenous infusion of 5 μg noradrenaline for 10 min. Records in (*b*) and (*c*) were taken 25 and 120 min respectively after infusion of noradrenaline. Time: 1 min.

In 2 reserpine-treated animals the brain and spinal cord were destroyed. Infusion of noradrenaline in these 2 experiments did not restore the response to eserine.

The effect of reserpine and dihydroxyphenylalanine on the pressor response to eserine. The slow intravenous infusion of dihydroxyphenylalanine in reserpine-treated animals also did not significantly change the response to eserine. In 7 experiments 3 mg of dihydroxyphenylalanine was slowly infused into the jugular vein of the reserpine-treated animals. Only in 2 out of 7 experiments was the hypertensive response to eserine restored after infusion of dihydroxyphenylalanine. In one experiment 6 mg of dihydroxyphenylalanine was infused, but the response to eserine did not reappear. Fig. 2 shows an experiment in which infusion of dihydroxyphenylalanine restored the response to eserine. The usual response to eserine in the reserpine-treated animal is shown in (*a*). Between (*a*) and (*b*) 2.5 mg dihydroxyphenylalanine was infused intravenously for 12 min and the record in (*b*) was taken 30 min thereafter. The slight hypertensive response to eserine gradually disappeared after 3 hr, as shown in (*c*).

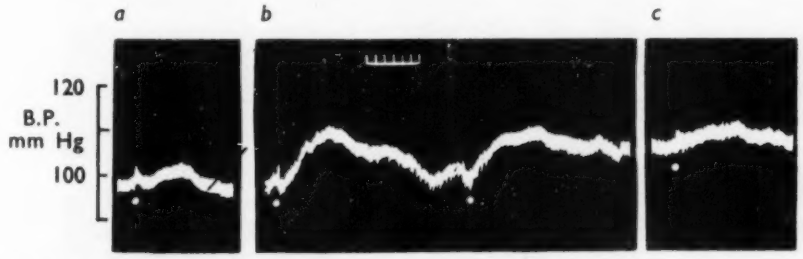

Fig. 2. The effect of eserine and slow intravenous infusion of dihydroxyphenylalanine on the blood pressure in a reserpine-treated rat (160 g). At white dots: 20 μg eserine intravenously. Between (*a*) and (*b*) 2.5 mg of dihydroxyphenylalanine was infused intravenously for 12 min. Records in (*b*) and (*c*) were taken 30 and 180 min respectively after infusion of dihydroxyphenylalanine. Time: 1 min.

The effect of reserpine and 5-hydroxytryptamine. In 7 reserpine-treated animals 5-hydroxytryptamine was infused intravenously in doses from 15 to 20 μg for 10 min. This infusion, however, did not restore the hypertensive effect of eserine. Only in 2 experiments was a slight potentiation observed.

The effect of reserpine and isopropylisoniazid. It has been found that pretreatment with isopropylisoniazid abolishes the effect of reserpine on the liberation of adrenaline from the adrenals (Kroneberg & Schümann, 1960). In the present experiments the animals were treated simultaneously by reserpine and by isopropylisoniazid in order to see whether the effect of reserpine on the response to eserine could be abolished by isopropylisoniazid. It was found that isopropylisoniazid did not abolish the inhibitory action of reserpine on the eserine hypertension. In another series of experiments isopropylisoniazid was injected after the animal was prepared for blood pressure recording. It was also found that isopropylisoniazid could not restore the hypertensive response to eserine once it was blocked by pretreatment with reserpine.

The effect of bretylium and eserine. Bretylium is a specific type of adrenergic blocking agent. It causes a specific and lasting depression of many excitatory and inhibitory responses evoked by electrical stimulation of the peripheral sympathetic nervous system, probably by impairing conduction of impulses in adrenergic neurones with consequent failure of noradrenaline and adrenaline release (Boura & Green, 1959). In our experiments bretylium abolished or significantly depressed the hypertensive response to eserine, and at the same time the hypertensive effect of adrenaline was unchanged or even potentiated. One experiment is shown in Fig. 3. The usual responses to eserine and adrenaline are shown in (*a*). Between (*a*) and (*b*) 10 mg/kg bretylium was injected intravenously and 30 min later the

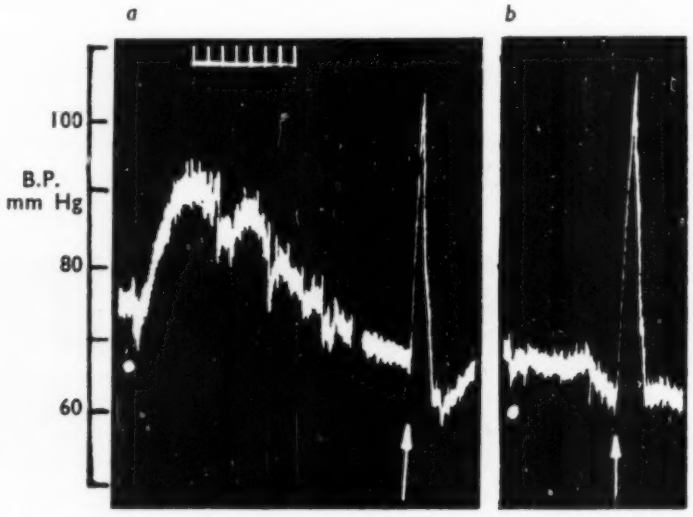

Fig. 3. The effect of eserine, adrenaline and bretylium injected intravenously on the blood pressure of the rat (190 g). At white dots: 20 μg eserine. At the arrows: 0.4 μg adrenaline. Between (*a*) and (*b*) 10 mg/kg bretylium. Record in (*b*) was taken 30 min after injection of bretylium. Time: 1 min.

response to eserine was completely blocked, while the effect of adrenaline was slightly increased, as shown in (*b*). It was also found that in doses which significantly depressed the action of eserine, bretylium did not inhibit the hypertension due to excitation of medullary centres induced by clamping the common carotid arteries.

Cocaine was found to reduce the inhibitory effect of bretylium on the hypertensive response to eserine. Boura & Green (1959) have already found that cocaine reduced the effect of bretylium on the response of the nictitating membrane to nerve stimulation. The antagonism between bretylium and cocaine on the blood pressure response to eserine is shown in Fig. 4. The usual hypertensive effect of eserine and adrenaline is shown in (*a*). Between (*a*) and (*b*) 9.2 mg/kg bretylium was injected intravenously and 30 min later the effect of eserine was abolished, while the response to adrenaline was unchanged, as shown in (*b*). Between (*b*) and (*c*) 3.7 mg/kg cocaine was injected intravenously and 10 min later the response to eserine was partly restored, as shown in (*c*).

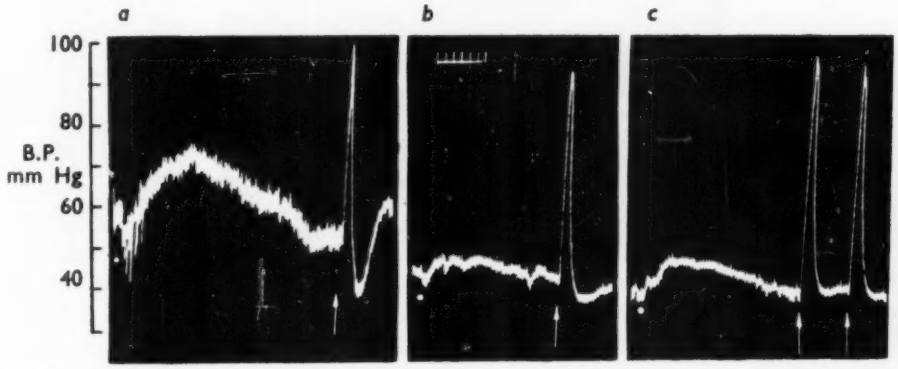

Fig. 4. The effect of eserine, adrenaline, bretylium and cocaine injected intravenously on the blood pressure of the rat (270 g). At white dots: 20 μg eserine. At the arrows: 0.4 μg adrenaline. Between (*a*) and (*b*) 9.2 mg/kg bretylium, and (*b*) was taken 30 min thereafter. Between (*b*) and (*c*) 1 mg cocaine, and (*c*) was taken 10 min thereafter. Time: 1 min.

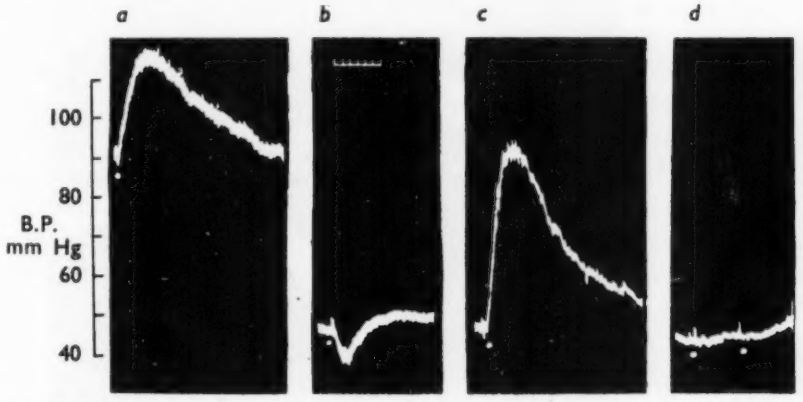

Fig. 5. The effect of hypothermia on the blood pressure response to eserine in the rat (250 g). (*a*) The response to eserine at normal body temperature. Records in (*b*) and (*d*) were taken at 21° C body temperature; (*c*) was taken at 26° C. At white dots: 20 μg eserine injected intravenously.

The effect of cooling. Following an observation of Dr. M. Miloševic (unpublished observations) that the hypertensive effect of some organophosphates was abolished by cooling, we have investigated the effect of cooling on the response to eserine. It was found that lowering of the body temperature by cooling the animal abolished completely the hypertensive effect of eserine. An experiment is shown in Fig. 5 where covering the animal with ice caused a reduction of body temperature, which was sometimes accompanied by a fall of blood pressure, as shown in Fig. 5(*b*). It can also be seen that the hypertensive effect of eserine was abolished at a body temperature of 21° C. The animal was then warmed and the body temperature rose to 26° C, at which temperature the hypertensive response to eserine reappeared, as shown in (*c*). The animal was cooled once again and the body temperature

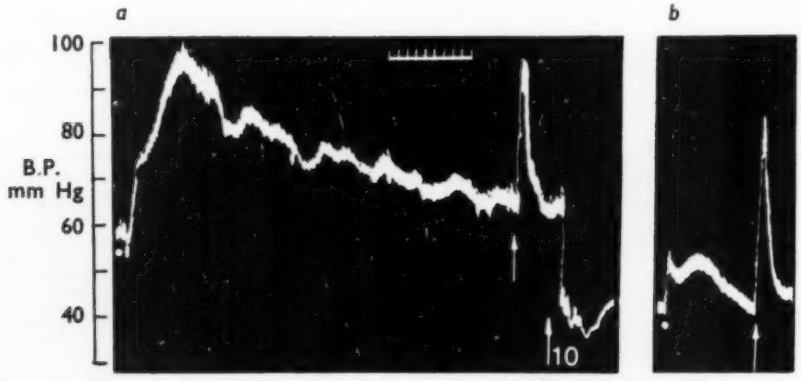

Fig. 6. The effect of eserine, noradrenaline and choline 2,6-xylyl ether bromide injected intravenously on the blood pressure of the rat (260 g). At white dots: 10 μg eserine. At the arrows: 1 μg noradrenaline. At the arrow with number 10: 7.7 mg/kg choline 2,6-xylyl ether bromide, and (*b*) was taken 34 min thereafter. Time: 1 min.

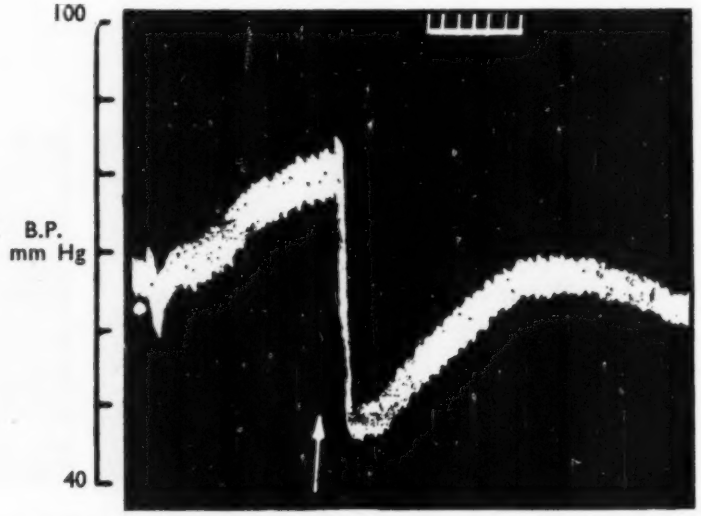

Fig. 7. The effect of eserine and choline 2,6-xylyl ether bromide injected intravenously on the blood pressure of the rat (250 g). At white dot: 20 μg eserine. At the arrow: 2 mg/kg choline 2,6-xylyl ether bromide.

dropped to 21° C with subsequent abolition of the hypertensive response to eserine, as shown in (*d*).

The effect of choline 2,6-xylyl ether bromide and eserine. In doses from 5 to 10 mg/kg choline 2,6-xylyl ether bromide significantly reduced the effect of eserine but not so readily as did bretylium. An experiment is shown in Fig. 6 where a particularly long lasting hypertensive effect of eserine is shown in (*a*). This effect can be compared with response to noradrenaline (at the first arrow). The intravenous injection of 7.7 mg/kg choline 2,6-xylyl ether bromide then caused a fall of blood pressure (at the second arrow). After 34 min the response to eserine was depressed, whereas the effect of noradrenaline was potentiated, as shown in (*b*). This effect of choline 2,6-xylyl ether bromide lasted for several hours. In one experiment choline 2,6-xylyl ether bromide was injected at the moment when the effect of eserine was still present. It was observed that the hypertensive response to eserine was blocked immediately after injection of choline 2,6-xylyl ether bromide (Fig. 7).

DISCUSSION

The present experiments show that the peripheral liberation of noradrenaline (and adrenaline) does not play a significant rôle in the hypertensive effect of eserine. In normal as in the reserpine-treated animals slow intravenous infusion of noradrenaline did not significantly alter the response to eserine. Similarly the infusion of dihydroxyphenylalanine as noradrenaline precursor in the reserpine-treated animals did not increase or restore the hypertensive effect of eserine. Thus, although eserine was found to increase the output of adrenaline from the adrenals of the cat (Stewart & Rogoff, 1921) it is unlikely that this effect occurs in the rat. The hypertensive response to eserine is different from that to tyramine. Burn & Rand (1958b) have found that the action of tyramine and substances acting like it can be restored in reserpine-treated animals by an infusion of noradrenaline into the blood stream. In the present experiments the hypertensive effect of eserine was regularly blocked by pretreatment with reserpine, but it was found that only occasionally and partially could it be restored by an intravenous infusion either of noradrenaline or dihydroxyphenylalanine.

Reserpine is known to liberate both noradrenaline and 5-hydroxytryptamine (Pletscher, Shore & Brodie, 1956 ; Shore & Brodie, 1957). We have now shown that the intravenous infusion of 5-hydroxytryptamine in reserpine-treated animals did not restore the hypertensive effect of eserine. It is known that pretreatment of the animal with isopropylisoniazid abolishes the effect of reserpine on the liberation of adrenaline from the adrenals (Kroneberg & Schümann, 1960). In the present experiments there was no antagonism between isopropylisoniazid and reserpine. The inhibitory effect of reserpine on the hypertensive response to eserine could not be reversed by pretreatment with isopropylisoniazid or by injection of isopropylisoniazid 30 to 180 min before eserine.

Lowering of the body temperature of the rat was found to abolish the hypertensive response to eserine. The present experiments do not allow any definite conclusion on the mechanism by which hypothermia abolishes eserine hypertension. It might be due to abolition of response of the nervous system since nervous elements

are known to be susceptible to lowering of temperature. It has also been found that the process of synaptic transmission is affected by low temperatures more readily than the process of depolarization of postsynaptic neurones (Varagic & Beleslin, 1958).

The experiments with bretylium and choline 2,6-xylyl ether bromide show that the hypertensive response to eserine is due more to adrenergic activation than to liberation of adrenaline and noradrenaline from the adrenals. Boura & Green (1959) have found that bretylium in concentrations which were able to block adrenergic neurones did not prevent the release of adrenaline and noradrenaline from the adrenal medulla by splanchnic nerve stimulation or by injection of dimethylphenylpiperazinium iodide. In the present experiments both bretylium and choline 2,6-xylyl ether bromide were found to depress or even block the hypertensive effect of eserine. Bretylium invariably had a stronger effect than choline 2,6-xylyl ether bromide. The possibility that the pressor effect of eserine might be blocked by a central action of bretylium is not supported by the experiments of Aviado & Dil (1960). They found that the intravenous injection of 2.5 to 20 mg/kg bretylium could not block the pressor response due to excitation of the medullary centres, induced either by cerebral ischaemia or by clamping the common carotid arteries. This response could be blocked, however, by combined adrenalectomy and injection of bretylium. We have now shown that bretylium, in doses which significantly depressed the pressor effect of eserine, did not inhibit the hypertension induced by clamping the common carotid arteries. It seems therefore that bretylium block of the eserine effect was most probably due to block of the adrenergic neurones. Nasmyth & Andrews (1959) have shown that cocaine antagonizes the adrenergic nerve block caused by choline 2,6-xylyl ether bromide, and Boura & Green (1959) found that cocaine antagonized the effect of bretylium. We have now shown that cocaine could partially restore the hypertensive response to eserine which was previously blocked by bretylium.

The results of the present experiments indicate that the hypertensive response to eserine in the rat is due to central activation of adrenergic nervous elements. The rôle of eserine in liberating noradrenaline and adrenaline from the adrenals and from the blood vessels is an insignificant factor in causing the hypertensive response to eserine.

We are indebted to Dr. A. F. Green, of the Wellcome Research Laboratories, for supplying bretylium, and to Dr. W. A. Bain, of Smith, Kline & French, for supplying choline 2,6-xylyl ether bromide.

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THE FORMATION OF AN ISOPRENALINE-LIKE SUBSTANCE FROM ADRENALINE

BY

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(*Received October* 26, 1960)

Intravenous injections of adrenaline, but not those of noradrenaline, caused the appearance of a substance which resembled isoprenaline in R_F value and pharmacological activity in blood withdrawn from the lower abdominal aortae of chloralosed cats rested after acute adrenalectomy and induction of lasting block in autonomic ganglia with hexamethonium. The formation of the isoprenaline-like compound was prevented by pretreatment with pyrogallol but not by pretreatment with reserpine, cocaine, phentolamine, dibenamine, harmaline, or iproniazid. The liver proved a main site of the origin of this isoprenaline-like substance.

Trace quantities of a substance which resembled isoprenaline in its pharmacological actions and R_F values were found in saline extracts of adrenal glands from cats, monkeys and man (Lockett, 1954); they appeared in blood perfusing cat heart–lung preparations when the upper thoracic sympathetic chains were stimulated (Lockett, 1957). Traces of a similar substance were detected in the plasma of blood withdrawn from the lower abdominal aortae of cats during pressor responses to intravenous injections of adrenaline; neither pretreatment of the animals with cocaine, 7 mg/kg subcutaneously, nor the intravenous injection of phentolamine, 2 mg/kg, prevented the appearance of the trace compound (Lockett, 1959).

The present work has confirmed and extended the hypothesis (Lockett, 1959) that the isoprenaline-like compound arises as a metabolite of adrenaline, and has shown that the liver is the major site of the formation of the metabolite. Conditions under which maximum yields of isoprenaline-like substance are obtained have been studied in expectation that the knowledge gained will lead to the isolation of this trace compound in quantity sufficient for its chemical identification.

METHODS

Anaesthesia was induced with ether and maintained by intravenous injection of chloralose 1.0%, 7 ml./kg, in male, female, or neuter cats. Tracheal and femoral venous cannulae were inserted, the adrenals were excluded from the circulation by purse-string ligatures and hexamethonium bromide was injected, 10 mg/kg both intravenously and subcutaneously, 30 to 40 min before every experiment. Heparin (Liquemin, Roche, 100,000 units/kg) was injected intravenously and the blood pressure was recorded from a carotid arterial cannula on all occasions. The quantities of hexamethonium used were regularly shown to block transmission of electrical excitation of an ascending cervical sympathetic chain to the corresponding nictitating membrane for the duration of the subsequent experiments.

In experiments designed for the study of the sympathetic amines present in lower aortic blood all injections were made into the right femoral venous cannula and blood samples were withdrawn through a polythene tube inserted into the aorta through the right femoral artery so that the tip lay 0.5 to 2.0 cm above the bifurcation. Ten minutes after the collection of a resting sample of arterial blood an intravenous injection of adrenaline or noradrenaline was made, and an arterial blood sample was collected throughout the response of the mean arterial pressure. The whole process was repeated 20 min after the effects of this injection had disappeared. The procedure differed when cocaine (7 mg/kg subcutaneously), phentolamine (2 mg/kg intravenously), dibenamine (20 mg/ml. propylene glycol, 1.0 ml./kg), harmaline (2.5 mg/kg intravenously), pyrogallol (25 mg/kg intravenously), or iproniazid (5 ml. 0.1 M in 0.9% NaCl/kg intraperitoneally) was used. The effect of the drug on the response of the mean arterial pressure to adrenaline was demonstrated before the first blood sample was collected. Animals pretreated with reserpine received 5 mg/kg of the hydrochloride (intramuscularly) 36 hr before anaesthesia was induced.

In experiments designed to demonstrate the major systemic site or sites of the liberation or formation of the isoprenaline-like substance, the procedure differed only in the following points. First, injections of adrenaline were made through polythene tubes inserted either into the hepatic artery (by retrograde cannulation of the splenic) or into the main splenic artery (by retrograde cannulation of a branch more centrally situated than the cannulated artery) or into a common iliac artery (by retrograde cannulation of the opposite femoral artery) or into the portal vein (by retrograde cannulation of the splenic vein and ligature of the splenic artery). Secondly, samples of venous blood were withdrawn in one experiment from the inferior vena cava by means of a polythene tube inserted through the right external jugular vein, the right atrium and thoracic inferior cava until the tip lay just above the entry of the hepatic veins. Alternatively, blood was withdrawn from a common iliac vein by retrograde cannulation of the opposite femoral vein. Electrical stimulation of the hepatic nerves was effected by means of shielded platinum electrodes delivering rectangular pulses of 0.5 msec duration at a rate of 20 per sec from a 2-volt source.

Blood samples of 5 to 8 ml. were collected into cooled heparinized tubes and the plasmas were separated without delay. Separate plasma pools were made from blood samples collected at rest and during responses to either adrenaline or noradrenaline.

The methods used to prepare protein-free extracts of plasma, for phenol-hydrochloric acid chromatography, and elution have been previously described (Lockett, 1957). The amines were separated chromatographically and assayed biologically in the eluates. Adrenaline was determined by inhibition of the response of the rat uterus to a fixed dose of acetylcholine, submaximal in effect (Gaddum, Peart & Vogt, 1949), and isoprenaline-like activity was assayed both by its depressor action on the arterial pressures of rats anaesthetized with pentobarbitone (Nembutal, Abbott Laboratories), 0.1 ml./100 g intraperitoneally, and on the rat uterus as described above.

Drugs. Heparin and iproniazid phosphate (Roche Products), reserpine and phentolamine (Ciba Laboratories), cocaine hydrochloride and adrenaline acid tartrate (Burroughs Wellcome), noradrenaline acid tartrate and harmaline (L. Light & Co.), isoprenaline hydrochloride (Bayer Products), hexamethonium bromide (May & Baker), pyrogallol (Hopkin & Williams), dibenamine (Smith, Kline & French Laboratories), and pentobarbitone sodium (Veterinary Nembutal, Abbott Laboratories) were obtained commercially.

RESULTS

No isoprenaline-like activity was demonstrable in plasma separated from blood withdrawn from the lower abdominal aortae of cats anaesthetized with chloralose which had been rested for 30 to 40 min after exclusion of the adrenal glands from the circulation and the induction of lasting block to transmission in autonomic ganglia. The resting plasma did, however, contain measurable concentrations of

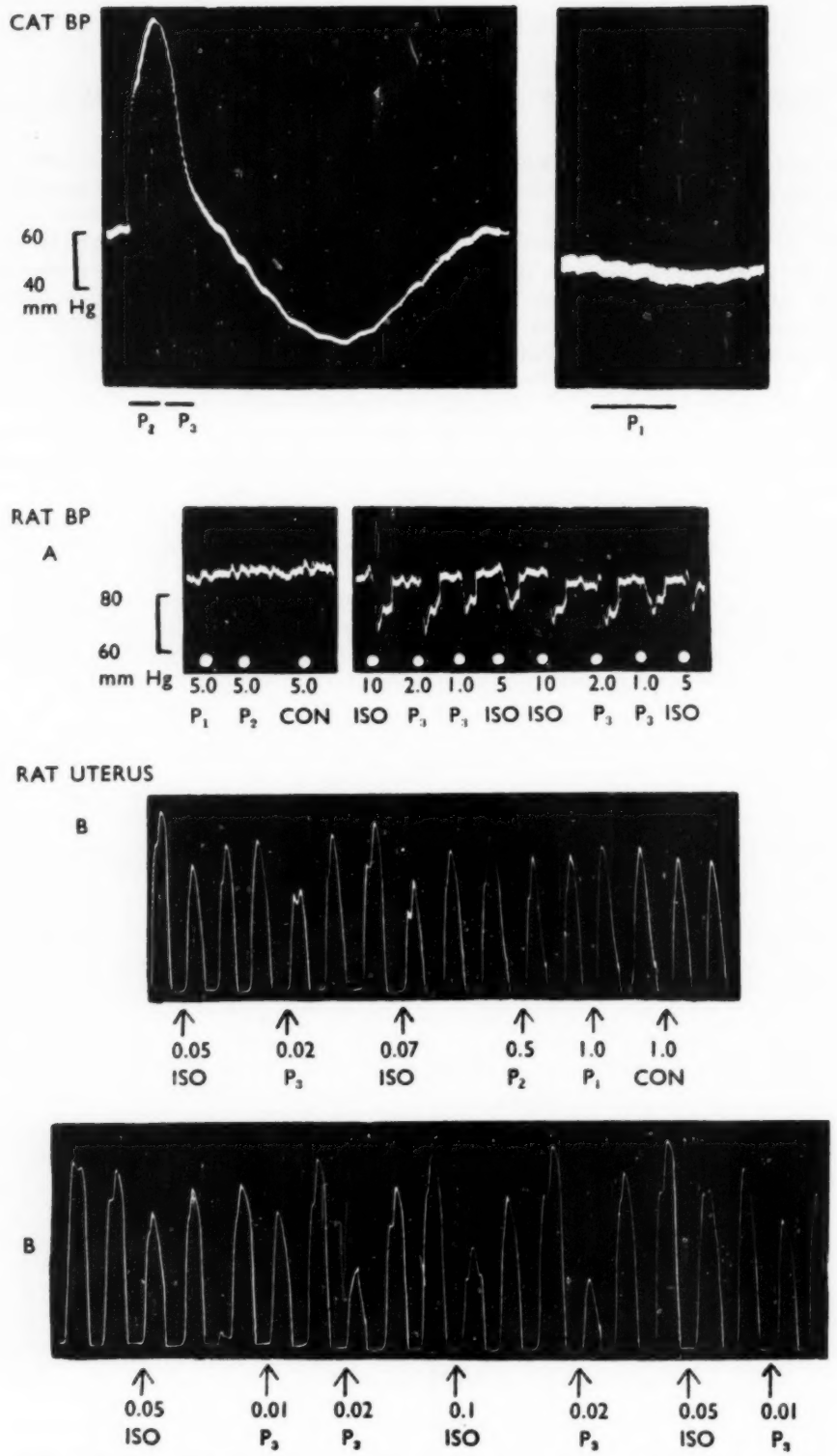

Fig. 1. Isoprenaline-like activity is demonstrable in the plasma of aortic blood taken from acutely adrenalectomized cats treated with hexamethonium during pressor responses to intravenous adrenaline. Above, record of the arterial pressure of cat during the collection of 10 ml. blood samples at rest (P_1) and during a response to 6 μg/kg adrenaline given intravenously (P_2 and P_3). Below, assays of isoprenaline-like activity separated chromatographically from the samples of cat plasma; A, by depression of the mean arterial pressure of a rat under pentobarbitone anaesthesia; B, by inhibition of submaximal effects of 0.2 μg acetylcholine on a quiescent rat uterus in 5 ml. aerated de Jalon fluid at 28° C. Doses: isoprenaline, ng (ISO); plasma eluates in ml. equivalent of original plasma (P_1, P_2, P_3); control eluate of R_F value intermediate between adrenaline and isoprenaline from plasma P_3 (CON).

adrenaline and noradrenaline (Lockett & Eakins, 1960). Isoprenaline-like activity (Fig. 1) appeared in the plasma during pressor responses to adrenaline but not during those to noradrenaline (Table 1).

TABLE 1

CONCENTRATIONS OF ISOPRENALINE-LIKE COMPOUND APPEARING IN PLASMA DURING PRESSOR RESPONSES TO INTRAVENOUS INJECTIONS OF ADRENALINE AND NORADRENALINE

The samples of blood were withdrawn from the lower aortae of cats anaesthetized with chloralose and rested for 30 to 40 min, after exclusion of the adrenal glands from the circulation and induction of lasting block in ganglia with hexamethonium

		Plasma concentration, μg/100 ml.					
		Adrenaline		Noradrenaline		Isoprenaline	
Treatment	No. of cats	Resting	Responding	Resting	Responding	Resting	Responding
Adrenaline (4–8 μg/kg)	(5)	0·07±0·01	1·1±0·25	—	—	<0·005	0·64±0·20
Noradrenaline (2·7–7 μg/kg)	(6)	—	—	1·68±0·69	10·7±2·05	<0·010	<0·005

Effect of inhibitors of the amine oxidase and O-methyl transferase on the concentrations of amines in aortic plasma. Pretreatment of cats with cocaine, pyrogallol, harmaline and iproniazid, in concentrations which increased the pressor effect of a fixed intravenous dose of adrenaline, increased the concentrations of adrenaline in the plasma of blood withdrawn from the lower abdominal aortae of the resting animals. Whereas isoprenaline-like activity became readily detectable at rest in the plasma of animals pretreated with cocaine, harmaline and iproniazid, only traces were found after pretreatment with pyrogallol. The normal increases in the concentrations of adrenaline and of isoprenaline-like activity in the lower aortic plasma of these preparations during pressor responses to intravenous adrenaline were also modified by pretreatment with these drugs. Whereas the monoamine oxidase inhibitors, harmaline and iproniazid, caused moderate excess in the adrenaline concentrations and great excess in the concentrations of isoprenaline-like activity found during pressor responses to adrenaline, the opposite occurred after pretreatment with cocaine (Table 2). By contrast, pyrogallol, which inhibits the *O*-methyl transferase, accentuated the normal rise in the plasma concentration of adrenaline and prevented the increase in isoprenaline-like activity with which this rise is normally associated (Table 2).

Effect of antiadrenaline drugs and pretreatment with reserpine on the concentrations of amines in aortic plasma. Pretreatment of cats with the antiadrenaline drugs phentolamine and dibenamine increased the concentrations of adrenaline and of isoprenaline-like activity in the plasma of blood withdrawn from the lower aorta both at rest and during pressor responses to intravenous adrenaline (Table 2). Resting levels of adrenaline and isoprenaline-like activity were also raised in cats which had received intramuscular reserpine 36 hr previously and in which the concentrations of pressor amines in saline extracts of the aortae were less than $\frac{1}{3}$ of those of aortae taken from control animals. In these reserpine-treated animals, the increases in the concentrations of isoprenaline-like activity

TABLE 2

THE EFFECTS OF PRETREATMENT OF CATS WITH VARIOUS DRUGS ON THE CONCENTRATIONS OF ISOPRENALINE-LIKE SUBSTANCE APPEARING IN THE PLASMA

Blood samples were withdrawn from the lower aortae during pressor responses to intravenous injection of 4–8 μg/kg adrenaline. Experimental conditions as in Table 1

Pretreatment	No. of cats	Plasma concentrations μg/100 ml. Adrenaline Resting	Adrenaline Responding	Isoprenaline Resting	Isoprenaline Responding
1. None	(5)	0·07±0·01	1·1±0·25	<0·005	0·64±0·20
2. Harmaline	(3)	1·05±0·06	2·3±0·50	0·27±0·20	8·40±3·60
3. Iproniazid	(2)	0·14±0·05	3·8±0·20	3·51±0·86	8·04±2·37
4. Cocaine	(3)	0·52±0·24	8·4±1·88	0·04±0·01	1·08±0·36
5. Pyrogallol	(2)	1·20±0·80	5·0±2·41	<0·03	<0·03
6. Phentolamine	(3)	0·44±0·01	3·6±0·47	0·06±0·01	2·10±0·80
7. Dibenamine	(3)	0·62±0·04	5·7±0·36	0·05±0·02	1·72±0·27
8. Reserpine	(3)	0·17±0·12	2·3±0·24	0·33±0·30	1·06±0·04

of the aortic plasma during pressor responses to adrenaline were not subnormal (Table 2).

A search for a major site of origin of the isoprenaline-like activity. Sites of origin of the isoprenaline-like activity were sought by measurement of change in its concentration in the plasma of venous blood from an organ or limb in response to injection of adrenaline into corresponding arteries. Whereas no isoprenaline-like activity could be detected in venous blood returning from a leg when adrenaline had been injected into the corresponding common iliac artery (Table 3), it appeared in the plasma of blood withdrawn from the inferior vena

TABLE 3

SITES OF ORIGIN OF THE ISOPRENALINE-LIKE COMPOUND

Changes in the concentration of isoprenaline-like activity in venous plasma in response to arterial injections of adrenaline 4–6 μg/kg. Experimental conditions as in Table 1

Site of injection of adrenaline	Site of plasma sampling	μg/Isoprenaline/100 ml. plasma Resting	Responding
Abdominal aorta	Iliac vein	<0·005 (2)	<0·005 (2)
Splenic artery	Thoracic cava just above entry of hepatic veins	1·54±0·02 (2)	2·75±0·18 (2)
Hepatic artery	Thoracic cava just above entry of hepatic veins	0·67±0·50 (3)	3·40±0·02 (3)

cava just above the entry of the hepatic veins in response to injections of adrenaline made either into the splenic or the hepatic arteries (Table 3). This indicated that the liver might function as a main source of isoprenaline-like activity. Comparison was therefore made of the concentrations of isoprenaline-like activity in plasma similarly withdrawn from the inferior vena cava at rest in response to the infusion of 5 μg adrenaline in 1 min either into the hepatic artery or into the portal vein. The mean concentrations of isoprenaline-like activity, expressed as μg isoprenaline/100 ml. plasma and followed by the standard errors of the means, found in three experiments were 0.05 ± 0.01 at rest, and 1.6 ± 0.26 and 4.3 ± 1.31 as a result of the infusions into the hepatic artery and portal vein respectively. Finally, the effect of stimulation of hepatic nerves on the release of

isoprenaline-like activity was examined. Changes in the concentrations of this activity in plasma from blood taken from the inferior vena cava just above the entry of the hepatic veins were again measured, but in response to stimulation of the hepatic nerves, to injections of adrenaline into a femoral vein, and to both procedures effected simultaneously. The results of the three experiments made are shown in Table 4. Stimulation of the hepatic nerves was alone sufficient to increase the concentration of isoprenaline-like activity in the caval plasma, and the changes induced by stimulation summed with those of intravenous adrenaline.

TABLE 4

EFFECT OF STIMULATION OF THE HEPATIC NERVES ON THE CONCENTRATION OF ISOPRENALINE-LIKE ACTIVITY

Samples of thoracic caval blood were collected during, and in the absence of, intravenous injection of 10 μg adrenaline. Experimental conditions as in Table 1

μg. Isoprenaline activity/100 ml. plasma				No. of expts.
Resting	Stimulation (a)	Adrenaline (b)	(a)+(b)	
0·035±0·06	0·64±0·16	2·67±1·58	3·85±0·81	3

DISCUSSION

The isoprenaline-like activity (Fig. 1) found in the plasma of blood withdrawn from the lower abdominal aortae of cats during pressor responses to adrenaline might have been released by the adrenaline from preformed stores or have been due to the formation of a metabolite of adrenaline which closely resembled isoprenaline in R_F value and pharmacological effect.

The first hypothesis is made unlikely by the following facts: first, no such stores of isoprenaline-like activity have been found in the lungs or blood vessels of cats (Lockett, unpublished observations). Secondly, the mechanism of the release from stores might be expected to be contractile or chemical. Yet noradrenaline, which closely resembles adrenaline both in contractile activity and in chemical structure, does not cause the appearance of an isoprenaline-like substance (Table 1), and block of the contractile effects of both adrenaline and noradrenaline with the antiadrenaline drugs phentolamine and dibenamine does not prevent the appearance of isoprenaline-like activity in response to the intravenous injection of adrenaline (Table 2).

The experimental results, however, support the view that the isoprenaline-like compound is a metabolite of adrenaline. In general, pretreatment with drugs which caused increase in the concentration of adrenaline in plasma withdrawn from the lower aorta during rest and supranormal increase in this concentration when adrenaline was injected intravenously also raised the resting and " responding " concentrations of isoprenaline-like activity in the plasma (Table 2). This was so, irrespective of the mechanism which occasioned the raised concentration of adrenaline in plasma except after pretreatment with pyrogallol, a compound which inhibits the *O*-methyl transferase (La Brosse, Axelrod & Kety, 1958 ; Bacq, Brown & Ferry, 1960). This last observation, coupled with the fact that the concentrations of isoprenaline-like substance were highest after pretreatment with the monoamine oxidase inhibitors harmaline and iproniazid (Udenfriend, Witkop,

Redfield & Weissbach, 1958), suggests that the *O*-methyl transferase system plays an essential part in the formation of the isoprenaline-like compound which, once formed, is destroyed by the monoamine oxidase. The fact that the plasma levels of the isoprenaline-like activity were raised disproportionately to those of adrenaline after pretreatment with reserpine may perhaps have resulted from inhibition of the absorption of adrenaline by blood cells (Hughes & Brodie, 1959) so that a greater proportion of the injected adrenaline became subjected to the action of the *O*-methyl transferase of liver and other tissues.

Not all tissues are capable of converting adrenaline to the isoprenaline-like compound (Table 3). The evidence indicates that the liver is a major site of its formation (Table 4) and the finding that stimulation of the hepatic nerves is alone sufficient to cause the appearance of isoprenaline-like activity in blood withdrawn from the thoracic portion of the inferior vena cava is consistent with previous observations (Lockett, 1957).

Several further points of interest arise from our experiments. The raised plasma levels both of adrenaline and isoprenaline-like compound found after pretreatment with reserpine support the view that reserpine not only liberates sympathetic amines from tissue stores (Bertler, Carlsson & Rosengren, 1956; Burn & Rand, 1958) but also prevents their absorption on blood cells (Hughes & Brodie, 1959). Similarly raised plasma levels of adrenaline after pretreatment with harmaline and iproniazid suggest circulating adrenaline is in part inactivated by the monoamine oxidase without prior *O*-methylation, or that the accumulation of *O*-methylated adrenaline resulting from inhibition of the monoamine oxidase slows activity of the transferase. The increased plasma concentrations of adrenaline after phentolamine and dibenamine in arterial blood supplement the observations of Brown & Gillespie (1957) and Brown, Davies & Gillespie (1958) made on venous blood. These authors suggested that the increase in the concentrations of amines in the venous blood organs during stimulation of the sympathetic supply which followed the use of antiadrenaline drugs might result from the absence of their utilization at receptor sites. More work on the mode of action of these drugs is needed before this sole explanation can be accepted with confidence.

This work was undertaken during the tenure by K. E. Eakins of an educational grant made by the Pharmaceutical Society of Great Britain.

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THE EFFECT OF ADRENALINE, NORADRENALINE AND ISOPRENALINE ON THE GUINEA-PIG UTERUS

BY

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(*Received November* 9, 1960)

The actions of adrenaline, noradrenaline and isoprenaline were compared on the isolated guinea-pig uterus. In uteri from immature animals (200 to 350 g) adrenaline caused relaxation which changed to a biphasic effect and finally to a contraction in the course of an experiment (6 to 8 hr). Noradrenaline always caused contraction and isoprenaline relaxation. In uteri from oestrogen-treated animals adrenaline and noradrenaline caused contraction and isoprenaline caused relaxation. Isoprenaline potentiated the contraction produced by adrenaline and reversed the adrenaline relaxation to a contraction. The change of the pharmacological action of adrenaline was not related to the Na^+ and K^+ content of the uterus, which remained constant throughout an experiment involving repeated application of the amines. Nor could it be related to a change in the glycogenolytic effect of adrenaline estimated by determinations of total glycogen of the muscle which, however, may not reflect a momentary change in rate of breakdown.

The effect of adrenaline and noradrenaline on the isolated uterus from the guinea-pig has been investigated by several workers, particularly by Holtz & Wölpert (1937) and later by Greeff & Holtz (1951). They found that adrenaline caused a relaxation in uteri from immature guinea-pigs or from adult guinea-pigs in anoestrus. This relaxation was changed to a biphasic response—a contraction followed by a relaxation—or a pure contraction, when the guinea-pigs had been treated with gonadotrophic hormones. They did not consider the possibility that the change in the pharmacological action of adrenaline, which they produced by hormonal treatment, might occur by itself during a single experiment in the course of 8 hr.

Lundholm & Mohme-Lundholm (1956) tried to relate the mechanical response of adrenaline with its metabolic action. They found no significant decrease in the glycogen content when adrenaline was applied to the guinea-pig uterus. However, when the uteri were divided in two groups, one in which adrenaline caused a relaxation and another in which it caused a contraction or had an indefinite effect, then there was a significant decrease in muscle glycogen in the first group (from an average of 183 mg % the glycogen fell by 27.4 mg %). They were unable to show any glycogenolytic effect of adrenaline when glucose was present in the nutrient solution and they concluded that the relaxing effect of adrenaline was due to formation of lactic acid in the muscular tissue.

The investigation to be described in this paper was carried out in order to compare the effect of adrenaline and noradrenaline with that of isoprenaline. Another aim was to find if factors other than hormonal treatment might produce

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the same changes in the action of adrenaline as those described by Holtz and his co-workers.

METHODS

In vitro *experiments on the guinea-pig uterus*

Virgin female guinea-pigs weighing 200–350 g were used. Immediately after killing and bleeding the animals the right horn of the uterus was removed and set up in a 20 ml. isolated organ bath at 35° C. The composition of the bathing solution was (mM): Na^+ 137, K^+ 5.9, Ca^{++} 2.5, Mg^{++} 1.2, Cl^- 134, HCO_3^- 15.5, $H_2PO_4^-$ 1.2 and glucose 11.5. It was aerated with a gas mixture of 97% O_2 and 3% CO_2; the *p*H was 7.3.

Mechanical records were obtained on a smoked drum by means of an isotonic lever with frontal-writing point and a load of 1 g/cm muscle.

Most of the experiments were carried out by using uteri from untreated immature guinea-pigs. A few animals were treated with oestradiol (Dimenformon, Organon) 1 μg/g intramuscularly 48 hr before starting the experiment. The stage of the oestrus cycle was determined by vaginal smears.

Sodium and potassium determinations

To determine the Na^+ and the K^+ content of the tissue before and after an experiment, one horn was taken for Na^+ and K^+ determination immediately after killing the guinea-pig, while the other horn was used for the determination at the end of the experiment. The fresh weight of the first horn was determined, but only the wet weight of the second horn was obtained at the end of the experiment. Both horns were transferred to small containers which were kept overnight in a drying oven at 105° C in order to determine the dry weight. In this way the ratio $\frac{\text{fresh wt.}}{\text{dry wt.}}$ of one horn and $\frac{\text{wet wt.}}{\text{dry wt.}}$ of the other horn could be calculated.

The pots containing the dried uteri were placed in small recesses in the top of a heavy steel block and to each pot was added 1 ml. concentrated Analar nitric acid and 1 ml. concentrated Analar perchloric acid. The temperature was then raised slowly to 150° C and in this way the uteri were ashed until no organic material was left. The residue was dissolved in distilled water. The Na^+ and K^+ content was determined by flame photometry with a Beckman DU flame photometer at a wavelength of 589 mμ and 769 mμ respectively and compared with standard solutions of NaCl and KCl.

Glycogen determinations

Many workers have found that in some species, for instance, rat (Walaas, 1952), a considerable part of the uterine glycogen is situated in the endometrium. As the present work was concerned with the glycogen content of the myometrium a preliminary experiment was carried out to see whether the glycogen distribution was the same in the guinea-pig and in the rat. A technique similar to that described by Walaas (1952) was used. Uteri were quickly dissected and transferred to a watch-glass placed on solid CO_2. The uteri from which the endometrium was to be removed were split longitudinally and the peritoneal side was frozen to the watch-glass. The endometrium was then scraped off with a scalpel. The remaining myometrium was quickly weighed and transferred to boiling potassium hydroxide for glycogen estimation which was carried out as follows. The uterine horns, either freshly removed from the body, or at the end of an experiment from the isolated organ bath, were weighed and placed in 10 ml. graduated centrifuge tubes containing 1 ml. 30% potassium hydroxide in a boiling water bath. After boiling for 15 min the tissue was usually dissolved completely and the cotton knots were then removed. The volume was made up to 5 ml. with 95% ethanol and the centrifuge tubes were kept overnight in a deep freeze at −20° C for glycogen precipitation. The next morning the glycogen was spun down for 5 min at 3,000 rev/min and the supernatant was discarded. The solid glycogen was hydrolysed to glucose by boiling it in 1 ml. 3N sulphuric acid for 2 to 3 hr. The glucose was estimated by the method of Huggett & Nixon (1957). This very sensitive method was necessary because the total amount of glycogen was only about 50 μg. Furthermore the method is claimed to be very specific, and this might account for the glucose values in this paper being rather small compared with those of other authors. The glycogen content was expressed in mg glucose per 100 g fresh wt. throughout.

Lundholm & Mohme-Lundholm (1956) found that there was a big animal variation in the glycogen content. In the present experiments the scatter was not so great, possibly because only immature guinea-pigs were used. Lundholm & Mohme-Lundholm (1956) used adult animals, and the scatter which they found might be connected with different stages of the oestrus cycle.

To avoid the effect of variation between animals the changes in the glycogen content caused by adrenaline were expressed as percentages by comparing the test horn with a control horn from the same animal. The right and the left horn were taken alternately as control and test object.

The experiments in which the glycogenolytic effect of adrenaline was determined were carried out as follows. Three isolated organ baths were set up and kept at the same temperature. The first bath contained the usual bathing solution. Both horns from each guinea-pig uterus were set up in this solution and stretched by 20% of their *in situ* length. After a predetermined period one horn of each pair was removed to the second bath, the other to the third bath, both containing glucose-free solution. To the third bath adrenaline was added 20 sec before the uterus was immersed to give a concentration of 1×10^{-6}. After 5 min both horns were removed at the same time and the glycogen was determined.

RESULTS

Mechanical effect of adrenaline, noradrenaline and isoprenaline

Effect of hormonal treatment. When a horn from the guinea-pig uterus was set up in the isolated organ bath it usually exhibited a spontaneous tone at first. This gradually declined, but, after several hours, most preparations showed a rhythm of activity, contracting and relaxing at regular intervals.

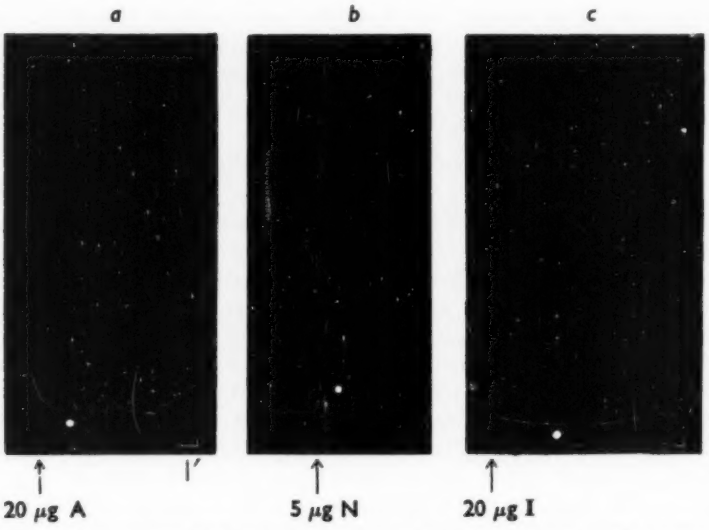

Fig. 1. The effect of adrenaline (A), noradrenaline (N) and isoprenaline (I) on the isolated uterus from an immature guinea-pig, recorded at the beginning of an experiment. In this and all subsequent tracings a dot indicates the time at which the amines were washed out. Time : 1 min.

The effects of adrenaline, noradrenaline and isoprenaline on an isolated uterus from an immature guinea-pig are illustrated in Fig. 1. The responses were obtained at the beginning of an experiment, when a slight spontaneous tone was present. Adrenaline caused a relaxation and noradrenaline caused a contraction. Isoprenaline caused a relaxation and it stopped the spontaneous activity.

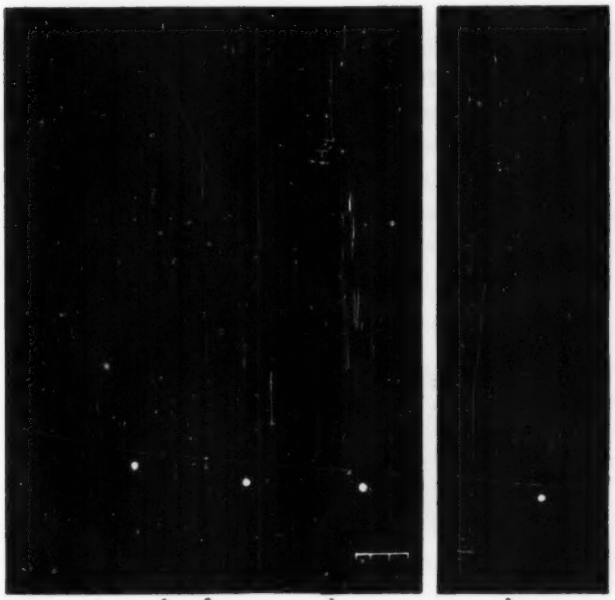

Fig. 2. The effect of adrenaline (A), noradrenaline (N) and isoprenaline (I) on an isolated uterus from a guinea-pig in oestrus. Time: 1 min.

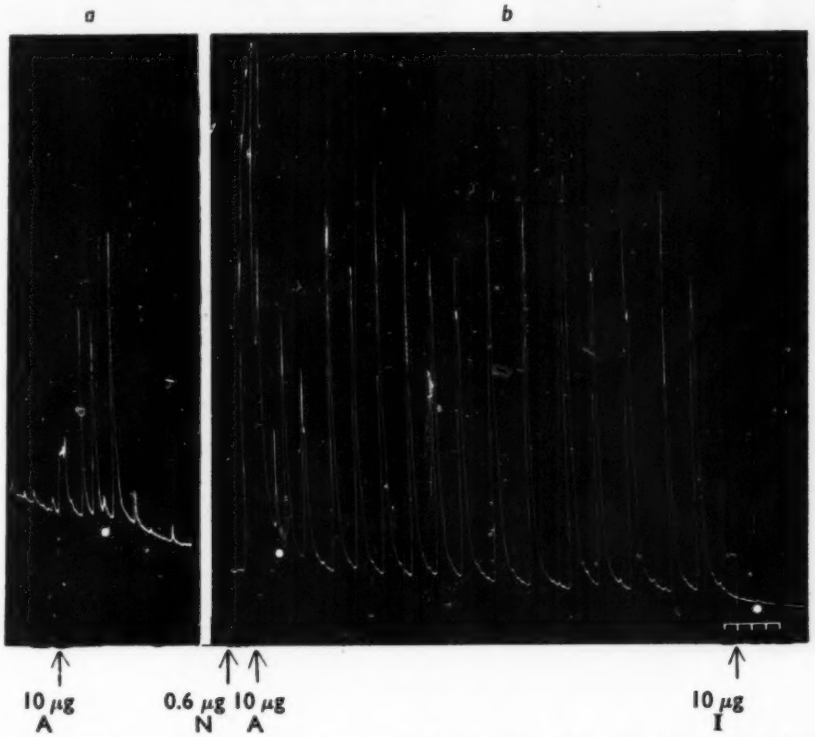

Fig. 3. Uterus from an oestrogen-treated guinea-pig. Rhythmic contraction initiated by adrenaline (A) and effects of noradrenaline (N) and isoprenaline (I). For description, see text. Time: 1 min.

Fig. 2 shows the responses to the same three amines of a uterus from a guinea-pig which had been treated with oestradiol. Both adrenaline and noradrenaline now caused a contraction, and isoprenaline had no visible effect because the preparation was at that time completely relaxed.

Adrenaline frequently initiated a rhythm of spontaneous activity in a uterus taken from a guinea-pig in oestrus. Fig. 3*a* shows a sequence of contractions produced by 10 μg adrenaline and an additional contraction on washing out. Fig. 3*b* shows a contraction produced by noradrenaline ; and while this was still

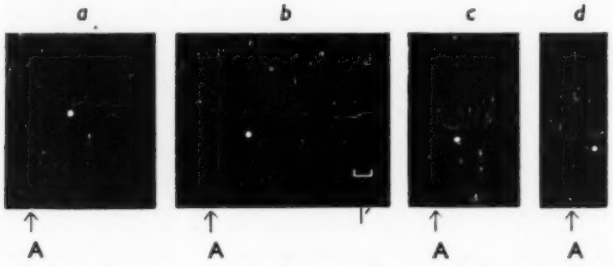

Fig. 4. Responses to 20 μg adrenaline (A) by the uterus from an immature guinea-pig. (*a*) 30 min, (*b*) 2 hr, (*c*) 3 hr and (*d*) 4 hr after the preparation was set up. Time: 1 min.

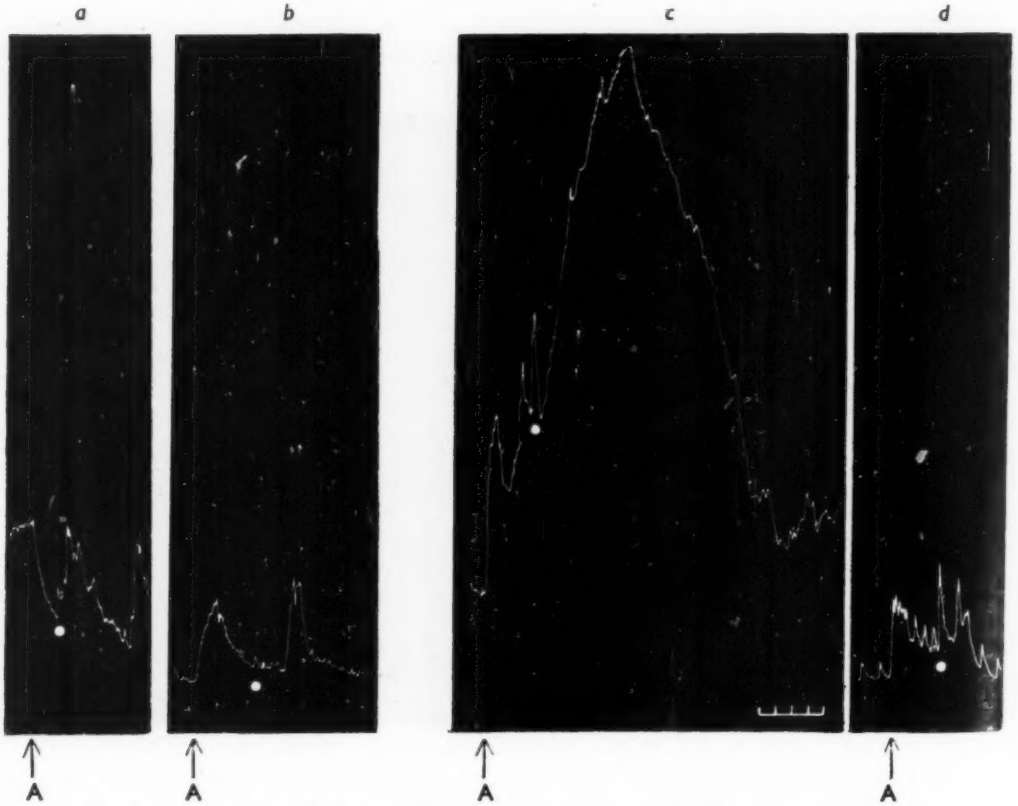

Fig. 5. Immature guinea-pig uterus. Response to 4 μg adrenaline (A) (*a*) 1 hr, (*b*) 4 hr after setting up the preparation on the first day ; (*c*) 30 min and (*d*) 2½ hr after setting up the same preparation on the second day, having been kept at 4° C overnight. Time: 1 min.

present the muscle relaxed. Adrenaline interrupted the relaxation causing a small contraction after which the relaxation continued. When both amines were washed out a regular rhythm of contractions started and continued for several min until isoprenaline was applied which stopped all activity.

Gradual change of the response to adrenaline in the course of an experiment. In six out of eight experiments on uteri from immature, untreated guinea-pigs a gradual change in the action of adrenaline was observed. At the beginning the response to adrenaline was a relaxation as shown in Fig. 4*a*. After some time this changed to a biphasic effect, usually a contraction followed by a relaxation (Fig. 4*b*). The biphasic effect was not observed in every experiment and occasionally the sequence was reversed, that is, a relaxation was followed by contraction. Often, while the muscle still relaxed in the presence of adrenaline, a big contraction was seen as an after-effect on washing out the adrenaline. During the later stages of an experiment the preparation responded to adrenaline by a contraction (Fig. 4*c* and *d*) or a series of contractions, unless a high spontaneous tone had developed.

A more marked change in the effect of adrenaline was noticed when it was applied to a preparation which had been kept overnight at 4° C. The responses shown in Fig. 5*a* and *b* were obtained from an immature guinea-pig uterus at the beginning and at the end of the first day. In Fig. 5*a* the preparation had a spontaneous rhythm which was interrupted by a relaxation when adrenaline was applied. In Fig. 5*b*, which was recorded several hr later on the same day,

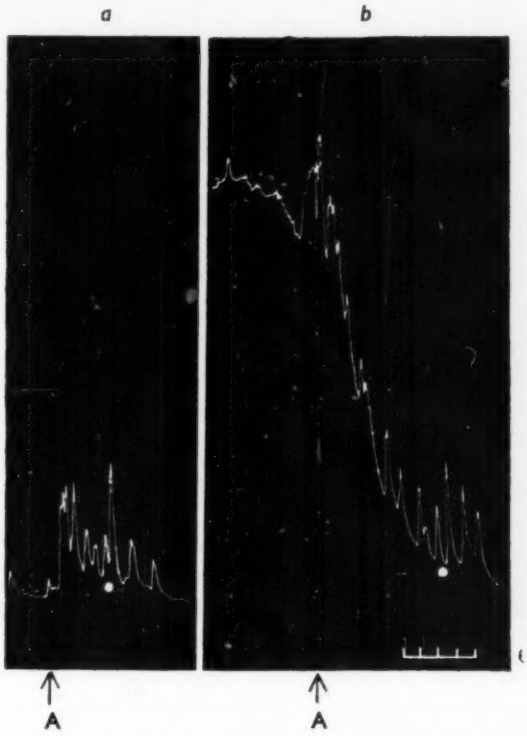

Fig. 6. Immature untreated guinea-pig uterus. 4 μg adrenaline (A) was applied when the tone was low in (*a*), when the tone was high in (*b*). Time: 1 min.

adrenaline caused a contraction. The records in Fig. 5*c* and *d* were obtained on the following day, after the preparation had been kept overnight at 4° C. Shortly after rewarming the uterus to 35° C it responded to adrenaline by a big contraction which was further increased when the adrenaline was washed out. Later in the same experiment adrenaline caused also a contraction, but this was smaller.

Dependence of the response to adrenaline on the tone. It was found that the effect of adrenaline depended on the tone which was present when it was applied. In Fig. 6 adrenaline was applied at first when the tone was low and it caused a contraction (*a*). Some time later when a high spontaneous tone had developed it caused a relaxation (*b*). Applied on this high tone adrenaline never caused a contraction, not even at the end of an experiment, unless isoprenaline had been given beforehand.

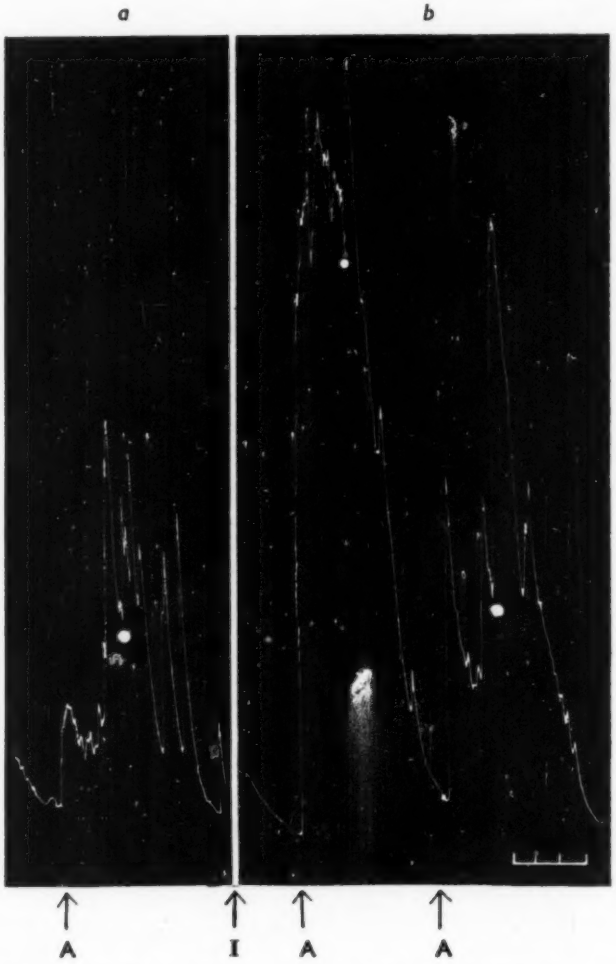

Fig. 7. The potentiation of the adrenaline contraction by isoprenaline. Contraction produced by 20 μg adrenaline (A) at (*a*) before and at (*b*) 5 min after 4 μg isoprenaline (I) had been added to the bath, and 4 min after both had been removed (see text). Time: 1 min.

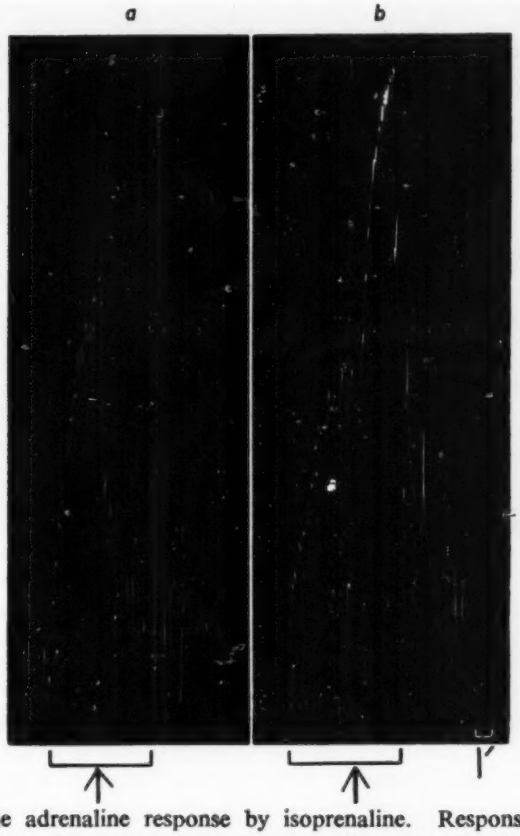

Fig. 8. Reversal of the adrenaline response by isoprenaline. Response of guinea-pig uterus to acetylcholine 5 μg, shown by the bars, and effect of adrenaline 20 μg (↑) on this contraction (*a*) and in the presence of isoprenaline (*b*). Time: 1 min.

Changes of the adrenaline effect caused by isoprenaline. Isoprenaline relaxed the uterus (Fig. 1). It also potentiated the contraction produced by adrenaline, and if adrenaline caused a relaxation this effect was reversed to a contraction when isoprenaline was present in the bath.

Fig. 7*a* shows a contraction caused by 20 μg adrenaline. Between 7*a* and *b* 4 μg isoprenaline was given. When adrenaline was applied 5 min later with isoprenaline still present the response to adrenaline was much greater. After washing out, the contraction initiated by adrenaline became smaller again.

The relaxing effect of adrenaline could be observed not only when the tone was spontaneously high, but also when it was produced by noradrenaline or by acetylcholine. In Fig. 8*a* adrenaline was given at the peak of a contraction caused by acetylcholine; it caused relaxation. Between *a* and *b* isoprenaline was added to the bath. To obtain the same size of contraction as before it was necessary to increase the dose of acetylcholine 30 times. When adrenaline was now applied, on the same level of contraction as before, it caused a further contraction instead of a relaxation.

Adrenaline also caused relaxation when it was given at the peak of a contraction produced by noradrenaline. In Fig. 9 noradrenaline was twice allowed to act for 3 min. The first contraction was cut short by giving adrenaline, while the second

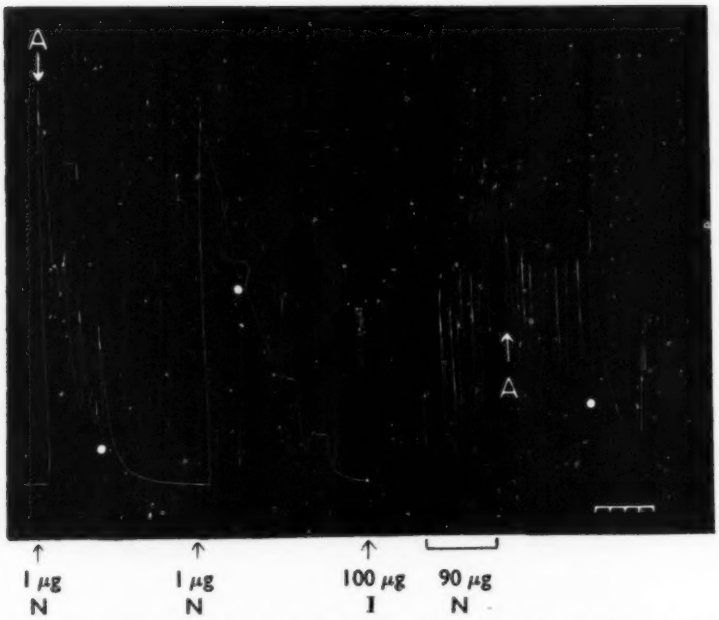

Fig. 9. The effect of isoprenaline on the relaxation caused by 10 μg adrenaline (A) when a tone was produced by noradrenaline (N). The first contraction was cut short by giving adrenaline at the peak. The second contraction was more maintained. In the presence of isoprenaline (I) adrenaline had no effect. For further description, see text. Time: 1 min.

contraction was more maintained. After the addition of isoprenaline the dose of adrenaline had to be increased 90 times and even then the contraction obtained was slower and smaller than before. When adrenaline was applied now it caused neither a contraction nor a relaxation.

One experiment was carried out using dichloroisoprenaline (1-(3′,4′-dichlorophenyl)-2-isopropylaminoethanol) in a concentration of 1×10^{-6}. It reversed a relaxation produced by 10 μg adrenaline to a large contraction.

Na^+ and K^+ content of guinea-pig uterus

For the Na^+ and K^+ estimations the fresh weight was determined in one horn which served as a control and the wet weight in the other horn which was used for the experiment. In Table 1, therefore, the Na^+ and K^+ content is expressed in mM/kg fresh wt. for the control muscle, while the corresponding value after 8 hr application of the amines is expressed in mM/kg wet wt. The figures are

TABLE 1

Na^+ AND K^+ IN GUINEA-PIG UTERUS (mM PER KG FRESH WT. AND WET WT. RESPECTIVELY ± S.D.)

Condition	Time after killing	dry wt. / fresh wt.	dry wt. / wet wt.	Na^+	K^+
In anoestrus	0	0·18±0·02		71·5±12·0	80·2±6·6
	8 hr		0·18±0·01	70·8±7·0	77·8±12·4
In oestrus	0	0·16±0·02		68·1±3·7	82·9±11·8
	8 hr		0·12±0·02	101·5±10·0	70·2±8·4

the mean of 8 determinations. The averages of the ratios $\frac{\text{dry wt.}}{\text{fresh wt.}}$ and $\frac{\text{dry wt.}}{\text{wet wt.}}$ were both 0.18 in the immature guinea-pigs in anoestrus. The table shows that after repeated applications of adrenaline, noradrenaline and isoprenaline in the course of an 8 hr experiment the Na^+ and K^+ content were the same as those in the initial control.

The same analysis was applied to the uteri from animals in oestrus and the results were entirely different. As seen from Table 1, the ratio $\frac{\text{dry wt.}}{\text{fresh wt.}}$ was 0.16 compared with 0.18 in the untreated animals. These results indicate that the water content was increased by the treatment with oestrogen although the difference was not significant. Futhermore the ratio $\frac{\text{dry wt.}}{\text{fresh wt.}}$ was reduced from 0.16 to 0.12 during the experiment indicating that the uteri had become oedematous. This difference was significant at the 5% level. Finally, although the K^+ content remained unchanged, the Na^+ content in oestrous uteri increased significantly ($P<0.001$) after repeated application of the amines. However, the uteri from the oestrogen-treated guinea-pigs were considerably heavier than those from untreated animals. Therefore the accumulation of Na^+ might be due to a larger degree of anoxia or possibly to Na^+-fixation in the connective tissue. On the other hand Goodford & Hermansen (1960) have shown that there was no accumulation of Na^+ in pieces of guinea-pig taenia coli kept in a poorly oxygenated organ bath compared with pieces in a well-oxygenated bath.

Glycogen determinations

Total glycogen content of uterine horns compared with that of the endometrium. In these experiments all the animals were treated with oestradiol 1 μg/g body weight given intramuscularly. The glycogen content in whole horns was 110.3 ± 9.5 mg %, while in the endometrium it was only about 15 mg %. This is less than 15% of the amount in the whole horn and was considered to be insignifi-

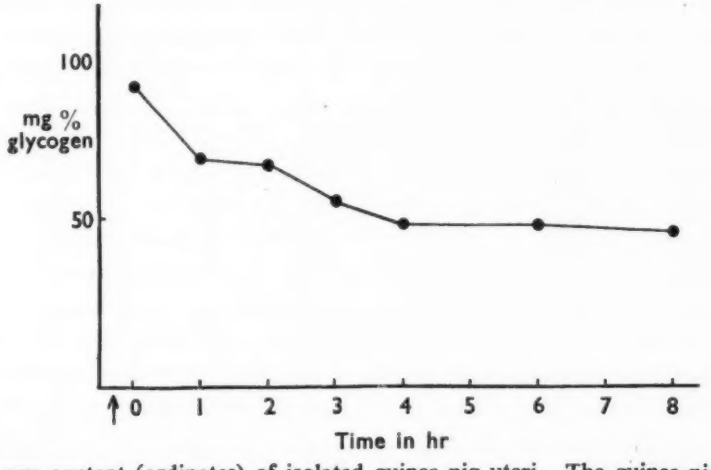

Fig. 10. Glycogen content (ordinates) of isolated guinea-pig uteri. The guinea-pigs were killed at a time marked by (↑) and the uteri set up in the organ bath at 0.

cant. Furthermore the loss of glycogen during the removal of the endometrium was an additional source of experimental error. Therefore all the estimations described were carried out on whole horns.

No comparison of the normal glycogen contents of the right and left horns was made. In order to avoid errors, if there existed an uneven distribution between the right and the left horn, they were taken alternately as controls. In order to avoid the effects of variation between animals, the experimental results were expressed relatively by comparing them with those of a control horn from the same guinea-pig.

Changes in the glycogen content during an experiment. In a few control experiments the amount of glycogen in uteri from seven untreated immature guinea-pigs kept for 1 to 8 hr in the usual bathing solution without any application of drugs was determined (see Fig. 10). The initial content immediately after killing the animal was about 90 mg %. During the first 4 hr in the isolated organ bath the glycogen content of the uteri gradually decreased to about 50 mg %. Between 4 and 8 hr *in vitro* there was apparently no further decline. The remarkable decrease of glycogen content occurred in the course of 8 hr in spite of the fact that no treatment was carried out and that external glucose was available in the normal concentration.

In a single experiment in which a preparation was exposed to glucose-free solution for 6 hr, the uterus was not completely depleted of glycogen, but 10% of the initial content was still present.

Glycogenolytic effect of adrenaline at the beginning and the end of an experiment. The finding that the glycogen content changed with the time in isolated conditions led to the investigation whether the glycogenolytic effect of adrenaline might also change during the course of an experiment. We therefore determined the change of the glycogen content produced by exposure to adrenaline 1×10^{-6} for 5 min in pieces kept for 30 min and for 8 hr in the isolated organ bath (Table 2). The mechanical effect was not recorded. The average effect of

TABLE 2

THE INFLUENCE OF ADRENALINE ON THE GLYCOGEN CONTENT (EXPRESSED IN MG % GLUCOSE) IN GUINEA-PIG'S UTERUS

	30 min in organ bath			8 hr in organ bath		
	Control	Adrenaline	±% of control	Control	Adrenaline	±% of control
	69·2	60·6	−12·5	71·4	76·8	+7·6
	56·5	60·2	+6·5	63·4	67·5	+6·5
	55·9	63·2	+13·1	74·4	73·8	−0·8
	77·5	74·0	−4·5	80·1	61·2	−23·6
	60·7	53·8	−11·4	73·2	71·1	−2·9
	75·3	72·7	−3·5	67·9	58·5	−13·9
	75·4	71·7	−4·9	72·2	69·4	−3·9
	66·3	59·5	−10·2			
Average	67·1	64·4	−4·1	71·8	68·3	−4·9

adrenaline was the same in both groups, that is, −4.1 and −4.9% respectively, but the action of adrenaline was not consistent. In both groups it caused an increase of the glycogen content in some and a decrease in other uteri. This confirmed the findings of Lundholm & Mohme-Lundholm (1956) that it was

impossible to show a significant change of the glycogen content after adrenaline, unless the uteri were separated into groups according to the mechanical reponse which adrenaline produced.

It must be pointed out that in Table 2 the controls removed after 8 hr had the same glycogen content as those removed after 30 min. This finding seems to disagree with the decline in glycogen content during an 8 hr experiment which has been described in the preceding section. However, the animals of the two groups in Table 2 were killed at different times of the day ; the 8 hr group at noon, the 30 min group between 3 and 7 p.m. Fluctuations in glycogen content may well occur during the day and the controls of the 2 groups are therefore not directly comparable.

DISCUSSION

Recently Axelsson, Bueding & Bülbring (1959) showed that in intestinal smooth muscle the relaxing effect of adrenaline coincided with an increased phosphorylase activity and they interpreted the adrenaline relaxation as a result of an accelerated supply of metabolic energy for processes stabilizing the membrane. If the same mode of action is assumed in uterine smooth muscle it might be possible to explain some of the observations described in this paper.

Ellis, Davis & Anderson (1955) found that isoprenaline in the rat diaphragm had a 10 times stronger glycogenolytic effect than adrenaline. If the same ratio existed in the guinea-pig uterus the phosphorylase activity might be very high in the presence of isoprenaline and adrenaline might then fail to cause a further increase. Hence it would fail to produce its inhibitory effect and the motor effect would become predominant. This is what was observed.

It is interesting that the di-chloro analogue of isoprenaline dichloroisoprenaline which has been investigated by Powell & Slater (1958) did not reverse the adrenaline-induced relaxation in the rat uterus. It is probably due to a species difference because they also found in a single experiment that dichloroisoprenaline could reverse the response of the guinea-pig uterus to adrenaline.

The reversal of the adrenaline effect by isoprenaline is complicated by the fact that isoprenaline itself relaxes the uterus, and that the stimulant action of adrenaline is more likely to appear when the tone is low. It was possible, however, with acetylcholine to bring the tone of the organ to the same level whether isoprenaline was present or not ; the reversal of the action of adrenaline by isoprenaline was then unequivocal. If noradrenaline was used to control the tone of the uterus, a different result was obtained ; in the presence of isoprenaline and sufficient noradrenaline to bring the tone to a standard level, adrenaline was now inactive. Under these conditions, the motor receptors were presumably saturated with noradrenaline, and the metabolic receptors with isoprenaline, so that adrenaline would have no scope for action.

On the basis of the hypothesis that relaxation is correlated with an accelerated glycogen breakdown and the finding that the amount of glycogen declined during the course of an experiment, it was tempting to speculate that there might be a causal connexion between the glycogen content and the change in the pharmacological action of adrenaline. However, no difference was found in the glycogenolytic effect of adrenaline between the beginning and the end of an experiment. From

these results it is not possible to deduce that the phosphorylase activity was also unchanged because glycogen estimations give only a very rough indication of this activity and further work is required to elucidate the change in the adrenaline effect.

The author is very indebted to Dr. Bülbring for her encouraging support, to Professor Paton for allowing him to work in his department and his interest in the work, to Dr. Blaschko for allowing him to use his Unicam spectrophotometer, and to Dr. J. Williamson, of the Department of Biochemistry, for advice and help with the method for determination of glycogen, and for supplying some enzyme solutions. He also wishes to thank Dr. Fakstorp, Pharmacia, Copenhagen, Denmark, who arranged his stay at Oxford.

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THE PERSISTENCE OF A DEPRESSOR RESPONSE TO OXYTOCIN IN THE FOWL AFTER DENERVATION AND BLOCKING AGENTS

BY

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(*Received November* 15, 1960)

The effects of oxytocin and vasopressin on the blood pressure of the fowl have been studied after surgical or chemical interruption of parts of the central and peripheral nervous systems, and after the administration of stilboestrol and progesterone. An augmented and prolonged depressor response to oxytocin was seen after atropine, bretylium, tetraethylammonium or decapitation, but not after decerebration, dihydroergotamine or dibenamine. There was a tendency for the pressor action of vasopressin to disappear after decapitation, decerebration, and all the blocking agents used, except tetraethylammonium. The differences between these results and those which have been obtained in rats are discussed.

It was shown in a recent paper (Lloyd & Pickford, 1961) that the effects of posterior pituitary hormones on the vascular system of the rat may be altered by chemical or surgical interruption of certain parts of the central and peripheral nervous systems. For example, although oxytocin was without effect on the blood pressure of the male or dioestrous female, despite dilatation of some vascular beds (Lloyd, 1959a), it acted as a pressor substance after pithing, decerebration, or sympathetic blockade at either the ganglionic or peripheral level. The pressor effect of vasopressin was greater than normal after such procedures.

A similar pressor response to oxytocin, and an increased response to vasopressin, were seen in the oestrous female, during late pregnancy, or after the administration of ovarian hormones to male or female rats (Lloyd, 1959a & b). It was therefore of interest to determine whether any changes in response to oxytocin and vasopressin could be induced by such procedures in other species. For this purpose the fowl was chosen, since a marked fall in blood pressure normally follows the injection of oxytocin, with little or no tachyphylaxis to small doses, and hence any conversion to a pressor response could be easily detected.

METHODS

White Leghorns of approximately 2 kg body weight were used, anaesthetized with sodium phenobarbitone (200 mg/kg) injected intramuscularly. All injections were made into a cannulated wing vein in a total volume of 0.4 ml. of 0.9% NaCl solution. Blood pressure was recorded on a kymograph from a mercury manometer connected to the cannulated ischiatic artery as described by Coon (1939). Sodium citrate (8% w/v) solution was used in the connecting pieces of the recording system. Stilboestrol dipropionate and progesterone (Progestin, B.D.H.) were injected subcutaneously in oil 24 or more hr before observations were

made. The oxytocin used was the synthetic brand Syntocinon (Sandoz) and the vasopressin was the Parke Davis product Pitressin. Occasionally the highly purified arginine vasopressin of du Vigneaud was employed. No difference was noticed in the effects of the commercial or highly purified extracts. The blocking agents used were bretylium tosylate (Darenthin, Wellcome Research Laboratories), dihydroergotamine (Sandoz), reserpine (Ciba Serpasil), dibenamine, and tetraethylammonium iodide. Decapitation was carried out by the method described for the duck by Paton (1912). Decerebration was carried out by opening the skull with a dental drill and bone forceps, reflecting the dura while leaving the longitudinal sinus intact, and removing the cerebrum by suction whilst an assistant compressed the carotid arteries against the transverse processes of the vertebrae. The cavity was then packed with gelatin sponge (Whitteridge, unpublished).

RESULTS

Normal hens

In this group the results were obtained from 20 hens, some of which were subsequently subjected to other procedures. In all cases oxytocin gave rise to an immediate and short-lasting depression of the blood pressure (Fig. 1A), and in any

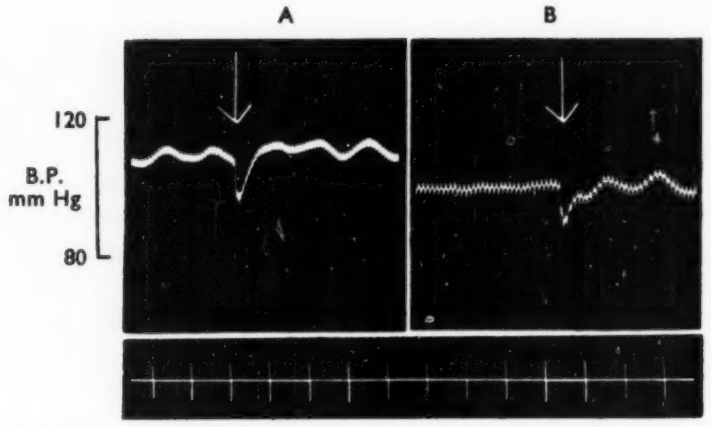

Fig. 1. The effect of 40 m-u. oxytocin on the blood pressure of the fowl (A) before and (B) after decerebration. Time, 30 sec.

one bird the magnitude and duration of this response remained constant, showing no hint of tachyphylaxis. In different birds, however, the dose needed to produce a depressor response of 10 mm Hg varied from 5 to 60 m-u. oxytocin. This may have been due, in part at least, to differences in basal blood pressure levels, which ranged from 85 to 115 mm Hg (mean 98 mm Hg). The type of response to vasopressin varied. In some cases a pure pressor response was obtained, and in others a transient depressor response, which might or might not be followed by a pressor response. In most instances 40 to 80 m-u. vasopressin was required to produce any alteration in blood pressure, but in some birds no response was obtained with up to 240 m-u. vasopressin. Whichever type of response was obtained, it tended to decrease during the course of the experiments, even when as long a period as 20 min was allowed between consecutive injections.

The effect of surgical interference with the nervous system

Decerebration. Decerebration was carried out on three hens after the normal responses to oxytocin and vasopressin had been established. In all three the blood pressure after decerebration was 5 to 10 mm Hg lower than before operation.

In all three experiments oxytocin remained depressor after operation, and in each case, allowing for the somewhat lower initial blood pressure, the magnitude and duration of the depressor response were unaltered (Fig. 1B). For example, in one bird, 40 m-u. oxytocin depressed the blood pressure by 10 mm Hg both before and after decerebration. In all three birds before operation, vasopressin caused a slight increase in blood pressure. After extirpation of the cortex, and giving the same dose of vasopressin, no pressor effect was seen ; instead there was a slight depression of 4 and 6 mm Hg respectively in two birds, and no response in the third (Fig. 2).

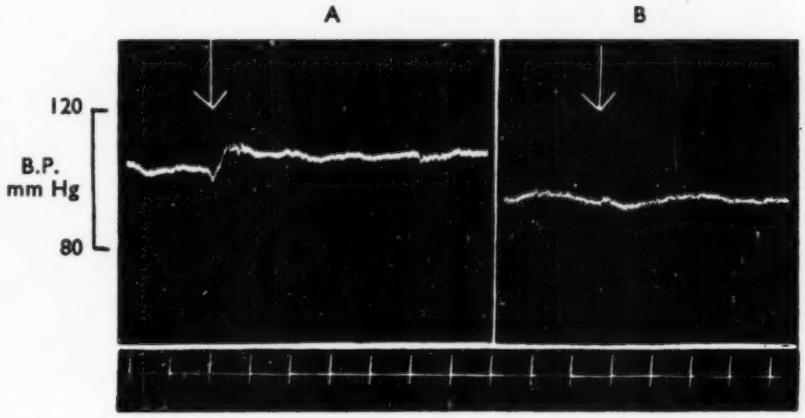

Fig. 2. The effect of 80 m-u. vasopressin on the blood pressure of the fowl (A) before and (B) after decerebration. Time, 30 sec.

Decapitation. A total of four hens was decapitated after the completion of control observations. After operation, the blood pressures were 70, 40, 50 and 45 mm Hg respectively, with the birds maintained on artificial respiration. In all four, oxytocin caused a fall in blood pressure both before and after decapitation (Fig. 3). In the bird in which the blood pressure was 70 mm Hg after operation, the depressor response to oxytocin was augmented and prolonged (Fig. 3 (i)), while in the birds with the lower blood pressures the response was prolonged, but reduced in magnitude (Fig. 3 (ii)). In all four after decapitation, vasopressin in doses of 80 m-u. caused a small depression in the blood pressure. This dose, before decapitation, had caused a similar fall in blood pressure in three of the hens, but a small rise in the fourth.

The effects of autonomic blocking agents

Atropine. Atropine, 1 to 2 mg, was administered intravenously to three normal hens after control observations were complete. This dose was sufficient to abolish all depressor responses to injected acetylcholine. The blood pressure was transiently elevated immediately following the injection of atropine, but rapidly returned to the normal level. In all three birds the depressor response to oxytocin was augmented

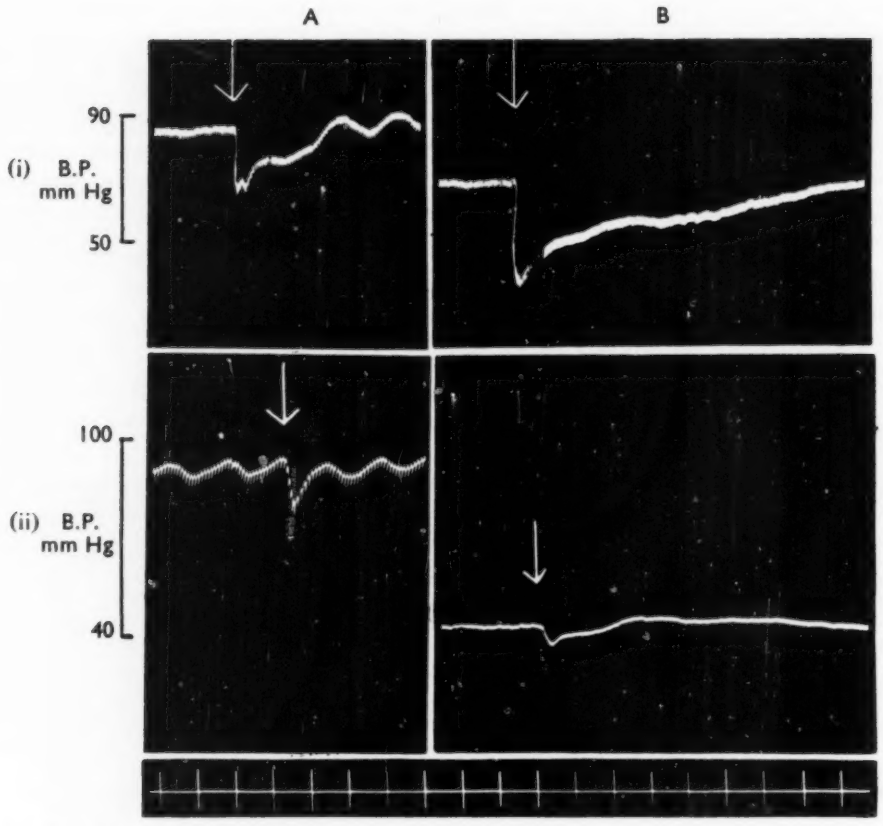

Fig. 3. The effect of 20 m-u. oxytocin on the blood pressure of two hens (A) before and (B) after decapitation. Time, 30 sec.

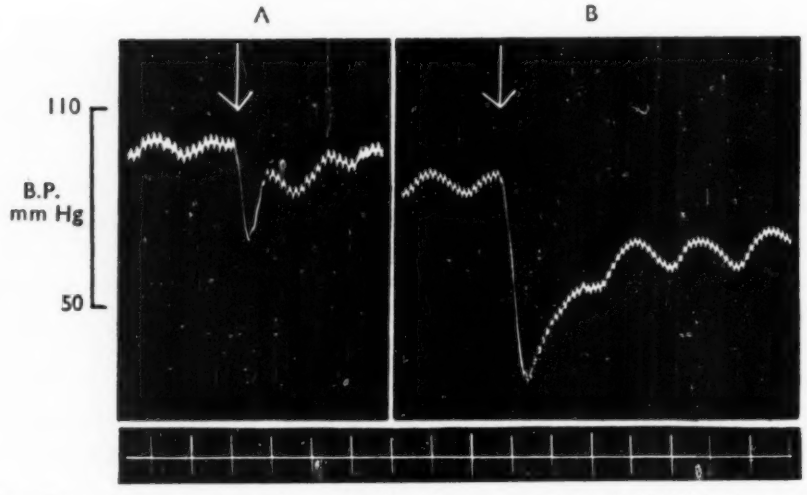

Fig. 4. The effect of 20 m-u. oxytocin on the blood pressure of the fowl (A) before and (B) after the intravenous injection of 1 mg atropine. Time, 30 sec.

and prolonged after atropine (Fig. 4). For example, in one hen 10 m-u. oxytocin caused a fall in blood pressure of 28 mm Hg before atropine, and a fall of 37 mm Hg after atropine. In two of the hens before treatment vasopressin had caused a diphasic response, a transient fall being followed by a slight but prolonged rise, while the third showed only the depressor phase of the response. After atropine, the pressor effect was reduced while the depressor phase was unaffected in the first two, and in the third the depressor effect was increased.

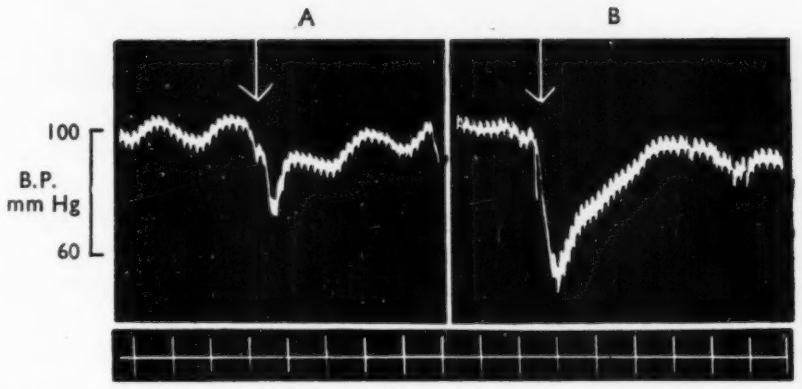

Fig. 5. The effect of 10 m-u. oxytocin on the blood pressure of the fowl (A) before and (B) after the intravenous injection of 6 mg bretylium tosylate. Time, 30 sec.

Bretylium tosylate. Bretylium tosylate (6 mg) was given intravenously to three hens after the normal vascular responses had been tested. In all three, after the blocking agent, the depressor response to oxytocin was increased and prolonged (Fig. 5). This increase in effect was noted immediately after the bretylium was given, and became maximal 50 to 60 min later, when the response was 50 to 55% greater than normal in all cases.

In one bird 80 m-u. vasopressin was without effect on the blood pressure either before or after bretylium. In the other two, a rise in blood pressure of 10 and 40 mm Hg respectively was produced by 80 m-u. before the administration of the blocking agent, and a fall of 40 mm Hg and a rise of 20 mm Hg respectively after bretylium.

Dihydroergotamine. Dihydroergotamine was given intravenously, in a dose of 1 to 1.3 mg, during observations on three hens. One of these had previously been treated with bretylium. The dose used was sufficient to abolish the pressor response to injected noradrenaline. In all three the depressor response to oxytocin was slightly reduced after the administration of the blocking agent (Fig. 6). For instance, 20 m-u. oxytocin gave a fall of 30 mm Hg in the blood pressure of one bird before blocking, and a fall of 25 mm Hg consistently afterwards. The small pressor response to vasopressin seen in all three birds when they were normal was absent after the administration of the dihydroergotamine.

Tetraethylammonium iodide. Three hens were treated with 8, 10, and 40 mg tetraethylammonium respectively. In all three the blood pressure fell some 5 to 10

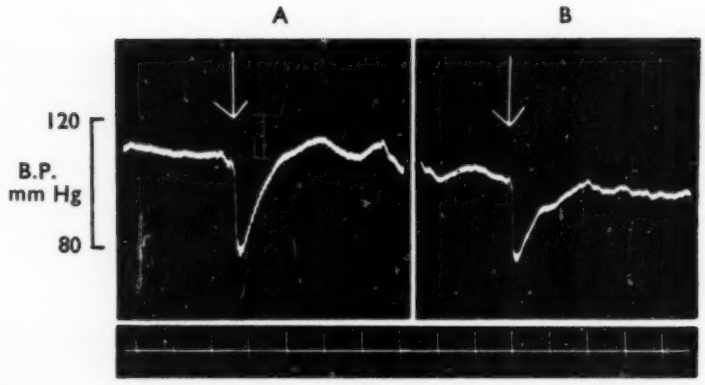

Fig. 6. The effect of 20 m-u. oxytocin on the blood pressure of the fowl (A) before and (B) after the intravenous injection of 1.3 mg dihydroergotamine. Time, 30 sec.

mm Hg below the pretreatment level. In one hen (which had received 40 mg tetraethylammonium) the depressor response to 20 m-u. oxytocin was only 6 mm Hg as compared with a fall of 28 mm Hg before tetraethylammonium. In the two which had received the smaller doses, the depressor responses to 20 m-u. oxytocin were increased from 28 to 34 mm Hg, and from 19 to 40 mm Hg respectively. In all three birds the small pressor response to vasopressin seen before tetraethylammonium administration was unchanged.

Dibenamine. Two hens were given dibenamine intravenously during the course of observations. In one bird the dose given was 20 mg, which was sufficient to abolish the pressor effect of injected noradrenaline, while in the other the dose was 5 mg, which reduced, but did not abolish, the adrenaline response. In both birds the blood pressure remained at the pre-injection level. In the bird given the larger dose of dibenamine the depressor response to 40 m-u. oxytocin was reduced from 16 mm Hg to 10 mm Hg, and in the bird given the smaller dose the fall in blood pressure in response to 20 m-u. oxytocin was reduced from 14 mm Hg to 10 mm Hg. In both birds before dibenamine, 80 m-u. vasopressin was slightly depressor, but after the blocking agent vasopressin had no effect at all on the blood pressure.

Reserpine. One hen was treated with 10 mg reserpine injected subcutaneously in divided doses on two consecutive days. On the following day the vascular responses were tested. No response was obtained to the injection of tyramine, while the responses to oxytocin and vasopressin did not appear to differ from those seen in untreated birds. 20 m-u. oxytocin gave a depression of the blood pressure of 10 mm Hg, while a slight depression was also seen in response to 80 m-u. vasopressin. The subsequent infusion of noradrenaline for 30 min at a rate of 2 μg/min did not affect the response to either oxytocin or vasopressin.

The effects of stilboestrol and progesterone administration

Stilboestrol dipropionate was injected subcutaneously or intramuscularly in five hens, in doses ranging from 50 to 100 μg/kg. This dose was more than adequate for the induction of the altered response to oxytocin in rats (Lloyd, 1959a). Observations were made 24 hr after treatment in four of the hens, and 48 hr after in the

fifth. In all five, oxytocin was depressor, 10 m-u. oxytocin giving a fall in blood pressure of 18 to 32 mm Hg. This appeared to be a larger response than that obtained with similar doses of oxytocin in normal hens, but the numbers are too limited to permit a more definite comparison. In all five birds vasopressin caused a preliminary fall in blood pressure, and in three of them this was followed by a pressor response. A sixth bird was treated with progesterone (1 mg), and a seventh given both stilboestrol (100 μg) and progesterone (1 mg). In these two birds the responses to both oxytocin and vasopressin were in every way similar to those seen in untreated birds.

DISCUSSION

The results described show that the dilator action of oxytocin in the normal hen remains dilator after extirpation of parts of the central nervous system, or ganglionic or peripheral blockade of the autonomic nervous system. This is in marked contrast to the rat, where a pressor and constrictor response to oxytocin was regularly seen after all such procedures, except the administration of atropine (Lloyd & Pickford, 1961). The persistence of the depressor response to pituitary extracts after decerebration in the hen has been described by Hogben & Schlapp (1924). In the present experiments the only marked differences induced in the action of oxytocin by any procedure were the prolongation (and in one instance augmentation) of the depressor response after decapitation, and a prolongation and augmentation after atropine, bretylium, and tetraethylammonium iodide. A similar effect has been noted in the duck after atropine by Paton & Watson (1912), who used whole posterior pituitary extract (Pituitrin), and in the chicken, again after atropine, by Coon (1939), who used the oxytocic fraction (Pitocin). All these workers attributed the greater and more prolonged effect following atropine to its ability to block a cardiac stimulating effect of the pituitary preparations. However, Woodbury & Abreu (1944) did not find that Pitocin augmented cardiac contraction in chickens. It is not possible to say whether a cardiac effect was concerned in the experiments described here, where the depressor response to oxytocin was increased and prolonged by very dissimilar agents and procedures, namely, atropine, bretylium, tetraethylammonium, and once after decapitation.

On the other hand dibenamine and dihydroergotamine somewhat reduced the depressor response to oxytocin. The only obvious difference between these two groups of agents is that noradrenaline is still constrictor in the presence of bretylium, tetraethylammonium and atropine, but is inactive following the administration of dibenamine or dihydroergotamine. It is wholly obscure how the possibility of a constrictor response to noradrenaline can be linked with an enhanced depressor response to oxytocin. No helpful information was provided by the use of reserpine, since this substance did not alter the response to either oxytocin or vasopressin, whether or not noradrenaline was infused. In this last respect at least, the chicken behaves like the rat (Lloyd & Pickford, 1961).

The effects of the procedures on the vascular responses to vasopressin were less well marked, but following decerebration, decapitation, and all the drugs used except tetraethylammonium, any pressor response previously seen was reduced or abolished, and in a few cases was even replaced by a depressor effect. In only a few experiments

did the normal response contain a depressor component, namely, three of the four experiments in which decapitation was performed, three experiments in which atropine was later given, and two in which dibenamine was given. After decapitation the depressor response was unchanged, after atropine it was in one case greater than before, and after dibenamine it was abolished. However, since vasopressin gives somewhat variable results in control experiments on normal birds, little can be said with certainty save that in no case was an increase in the pressor response to vasopressin seen. This is again in contrast to the rat, where the response to vasopressin was almost invariably increased after surgical or chemical denervation. The administration of stilboestrol, alone or in combination with progesterone, caused, if anything, a greater depressor response to oxytocin, and certainly no increase in the pressor effect of vasopressin, although the doses of ovarian hormones used were the same as, or greater than, those which in the rat regularly sensitized the animal to vasopressin and caused oxytocin to become a pressor substance (Lloyd, 1959a). It is not known, however, whether stilboestrol and progesterone would of themselves produce any vascular effects in chickens as they do in rats and many other mammals, or at what site such actions would occur.

Summarizing, the action of oxytocin on the blood pressure in the fowl is unchanged by decerebration, and slightly reduced by substances with properties like those of dibenamine and dihydroergotamine, but its depressor effect is greater and more prolonged after decapitation, or the administration of bretylium, tetraethylammonium, or atropine. In contrast, the pressor component of the action of vasopressin is reduced by all procedures except giving tetraethylammonium. If it were not for this exception it might be suggested that the pressor activity of vasopressin is in some way dependent on the connexion of the blood vessels with the nervous system. In most respects the results obtained in the fowl are the exact reverse of those found in the rat (Lloyd & Pickford, 1961).

Our thanks are due to Professor V. du Vigneaud for a gift of highly purified vasopressin ; to Dr. D. A. Long of the Wellcome Foundation for a gift of bretylium tosylate ; also to the United States Air Force (contract AF61 (052)-272) for financing this research. We also wish to thank Miss Helen Boyle for her invaluable technical assistance.

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